

Article

# Early Succession Patterns of Benthic Assemblages on Artificial Reefs in the Oligotrophic Eastern Mediterranean Basin

Ioannis Rallis <sup>1,2</sup> , Giorgos Chatzigeorgiou <sup>1</sup> , Marta Florido <sup>3</sup>, Francisco Sedano <sup>3</sup>, Avgi Procopiou <sup>4</sup>, Melina Chertz-Bynichaki <sup>5</sup>, Emmanouela Vernadou <sup>1</sup>, Wanda Plaiti <sup>1</sup>, Panayota Koulouri <sup>1</sup> , Costas Dounas <sup>1</sup> , Vasilis Gerovasileiou <sup>1,6,\*</sup>  and Thanos Dailianis <sup>1,\*</sup> 

- <sup>1</sup> Hellenic Centre for Marine Research (HCMR), Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), 71500 Heraklion, Greece; i.rallis@hcmr.gr (I.R.); chatzigeorgiou@hcmr.gr (G.C.); e.vernadou@hcmr.gr (E.V.); wanda@hcmr.gr (W.P.); yol72@hcmr.gr (P.K.); kdounas@hcmr.gr (C.D.)  
<sup>2</sup> Department of Biology, School of Sciences & Engineering, University of Crete, 71500 Heraklion, Greece  
<sup>3</sup> Laboratorio de Biología Marina, Departamento de Zoología, Facultad de Biología, Universidad de Sevilla, 41012 Seville, Spain; mflorido.c@gmail.com (M.F.); f.sedano.vera@hotmail.com (F.S.)  
<sup>4</sup> School of Sciences, European University Cyprus, Nicosia 1516, Cyprus; avg\_i\_procopiou@hotmail.com  
<sup>5</sup> Department of Marine Sciences, University of the Aegean, 81100 Mytilene, Greece; bynichakimelina@hotmail.com  
<sup>6</sup> Department of Environment, Ionian University, 29100 Zakynthos, Greece  
\* Correspondence: vgerovas@ionio.gr (V.G.); thanosd@hcmr.gr (T.D.)

**Abstract:** The colonization of artificial structures by benthic organisms in the marine realm is known to be affected by the general trophic patterns of the biogeographical zone and the prevailing environmental traits at the local scale. The present work aims to present quantitative data on the early settlement progress of macrofaunal benthic assemblages developing on artificial reefs (ARs) deployed at the Underwater Biotechnological Park of Crete (UBPC) in the oligotrophic Eastern Mediterranean. Visual census and subsequent image analysis combined with scraped quadrats were used to describe the establishment of the communities and their development over three consecutive campaigns, spanning 5 years post-deployment. Macroalgae consistently dominated in terms of coverage, while sessile invertebrates displayed different patterns over the years. Polychaeta and Bryozoa were gradually replaced by Cnidaria, while Porifera and Mollusca displayed an increasing trend over the years. Motile benthos was mainly represented by Mollusca, while the abundance of Polychaeta increased in contrast to that of Crustacea. For both sessile and motile assemblages, significant differences were observed among the years. The results of this study indicate that ecological succession is still ongoing, and further improvement in the monitoring methodology can assist towards a more accurate assessment of the community composition in complex AR structures.

**Keywords:** artificial habitats; sessile benthos; motile benthos; invertebrates; macroalgae; colonization; visual census; photoquadrats; scientific diving; Aegean Sea



**Citation:** Rallis, I.; Chatzigeorgiou, G.; Florido, M.; Sedano, F.; Procopiou, A.; Chertz-Bynichaki, M.; Vernadou, E.; Plaiti, W.; Koulouri, P.; Dounas, C.; et al. Early Succession Patterns of Benthic Assemblages on Artificial Reefs in the Oligotrophic Eastern Mediterranean Basin. *J. Mar. Sci. Eng.* **2022**, *10*, 620. <https://doi.org/10.3390/jmse10050620>

Academic Editor: Azizur Rahman

Received: 9 March 2022

Accepted: 26 April 2022

Published: 2 May 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

An ongoing anthropogenic modification and enhancement of European coastal marine ecosystems has taken place since the pioneering deployments of artificial reefs (ARs) in the 1960s [1,2], and most interventions are located in the Mediterranean Sea [3]. In Greece, the first AR initiative was established in 1999 in the North Aegean Sea [4], and since then, several AR installation initiatives and studies have followed [1,3,5]. ARs are usually deployed to serve the protection, restoration, and enrichment of marine ecosystems by: (a) acting as nurseries for certain marine taxa, (b) increasing the heterogeneity and variety of the seabed, (c) facilitating recreational fisheries, (d) supporting aquaculture, (e) enhancing recreational diving spots, and (f) supporting experimental marine research [1,6,7].

The Cretan Sea (NE Mediterranean), as part of the South Aegean ecoregion, has been characterized as strongly oligotrophic with limited primary productivity [8,9], high salinity

(often exceeding 39 PSU), and temperatures ranging from 14 to 28 °C throughout the year [10,11]. Artificial habitats with several heterogeneous microhabitats and structural refugia can attract various benthic and benthopelagic organisms [12]. Hence, ARs have become an important tool for marine life and biodiversity enrichment [13]. It is therefore interesting to study the long-term colonization and transformation of the macrobenthic assemblages that are established on ARs over the years, especially when monitoring is initiated concurrently or quickly after their installation. However, while AR-associated macrobenthic assemblages have been extensively studied in the Central and Western Mediterranean, similar assessments are limited in the oligotrophic regime of the Eastern Basin [1,7,14,15].

Different methods are used for sessile and motile benthic assemblages to study their ecological succession on ARs. For sessile communities, non-destructive methods (visual census monitoring with photoquadrats) have been proposed and are widely applied [16–19]. Motile communities, on the other hand, require sampling of the substrate surface with exhaustive methods by scraping and collecting the surface biotic layer, usually using diving. Therefore, Chatzigeorgiou et al. [20] proposed a hand-operated suction sampler (MANOSS) based on a slurp gun design, which is inexpensive, easy to construct, and allows the collection of successive samples through interchangeable collection mesh bags. The two approaches are proposed in a complementary manner for the characterization of benthic assemblages [21].

This study constitutes a long-term assessment of the structure and succession patterns of benthic communities developed on recently deployed ARs. Both non-destructive and scraping methods have been applied to evaluate the biodiversity patterns on sessile and motile components of benthic assemblages colonizing artificial structures over a 5-year time frame. The environment of their installation is in an experimental underwater facility located in the Southeastern Mediterranean.

## 2. Materials and Methods

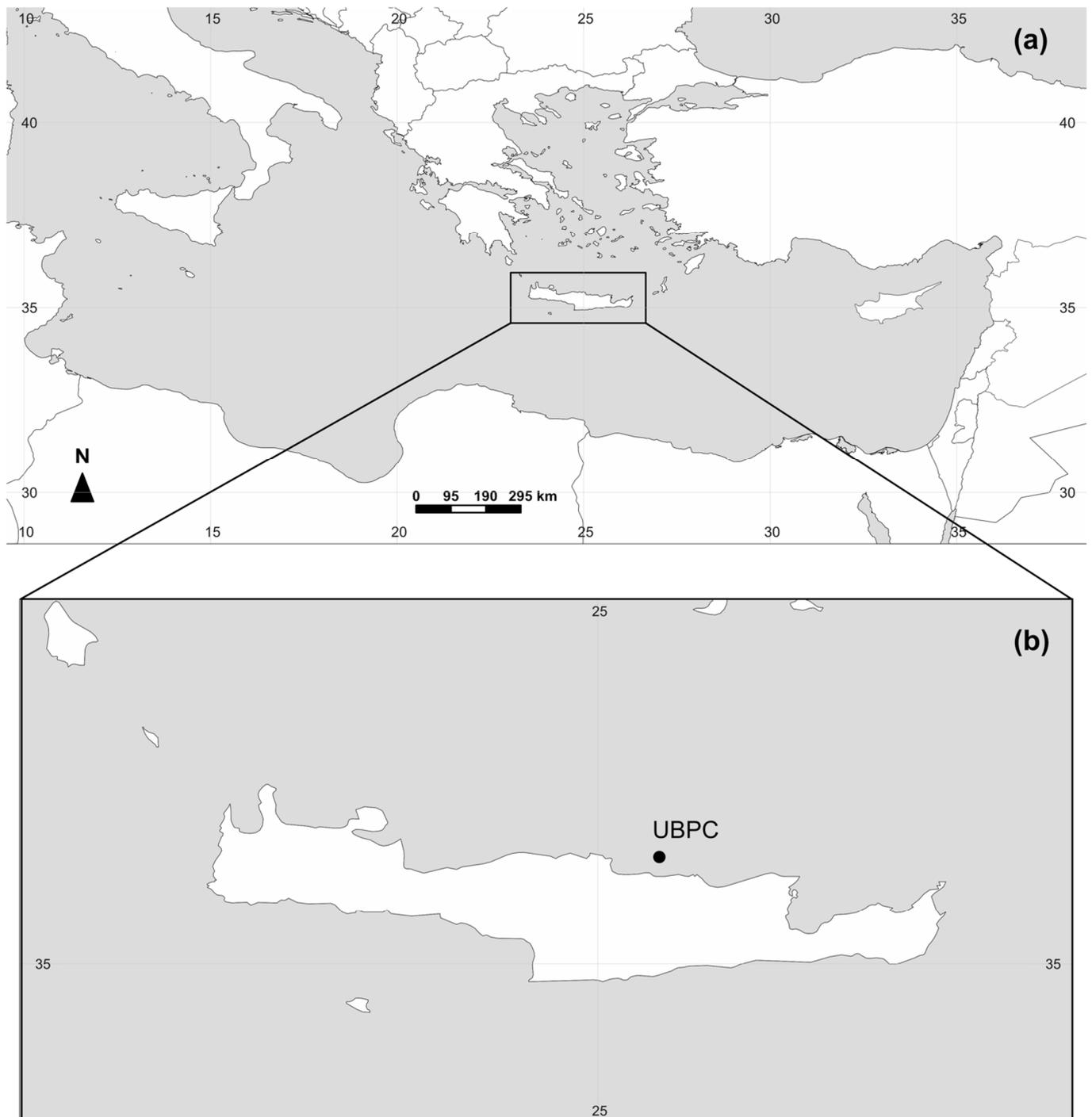
### 2.1. Study Area

The Underwater Biotechnological Park of Crete (UBPC) is an experimental underwater research infrastructure located at 1 nm from the northern Cretan coast, close to the land premises of the Hellenic Centre for Marine Research (HCMR) in Crete (<http://ubcrete.hcmr.gr/>, accessed on 11 April 2022). It comprises a seafloor area of 2.5 ha at a depth increasing from 18 to 22 m along the south–north direction (Figure 1) [12,22]. The seawater temperature at the area of the UBPC ranges from 14 to 28 °C over the year, while the salinity is relatively stable at 39 PSU, except for occasional dropdowns during the rainy season (November–April); the main current has a west to east direction due to prevailing NNW winds [11]. The seafloor area is characterized by a rather flat relief, intermixing coarse sand flats, and dead *Posidonia oceanica* mat, with a macroalgal cover mainly of *Caulerpa prolifera*. Patchy seagrass meadows (*P. oceanica*) are common along the area, while some natural small, flat, rocky reefs of less than a couple square meters of surface area are scarcely distributed. In 2015, twelve units of ARs made of concrete, with base diameters between 1.2 and 2.3 m and 1.4 to 2.5 m high, were deployed at a depth of ca. 20 m at UBPC. The purpose of the ARs project was to test the concept of a “recreational diving oasis” in order to attract diving tourism in overexploited and oligotrophic coastal ecosystems by mimicking natural rocky reefs [12,22] (Figure 2).

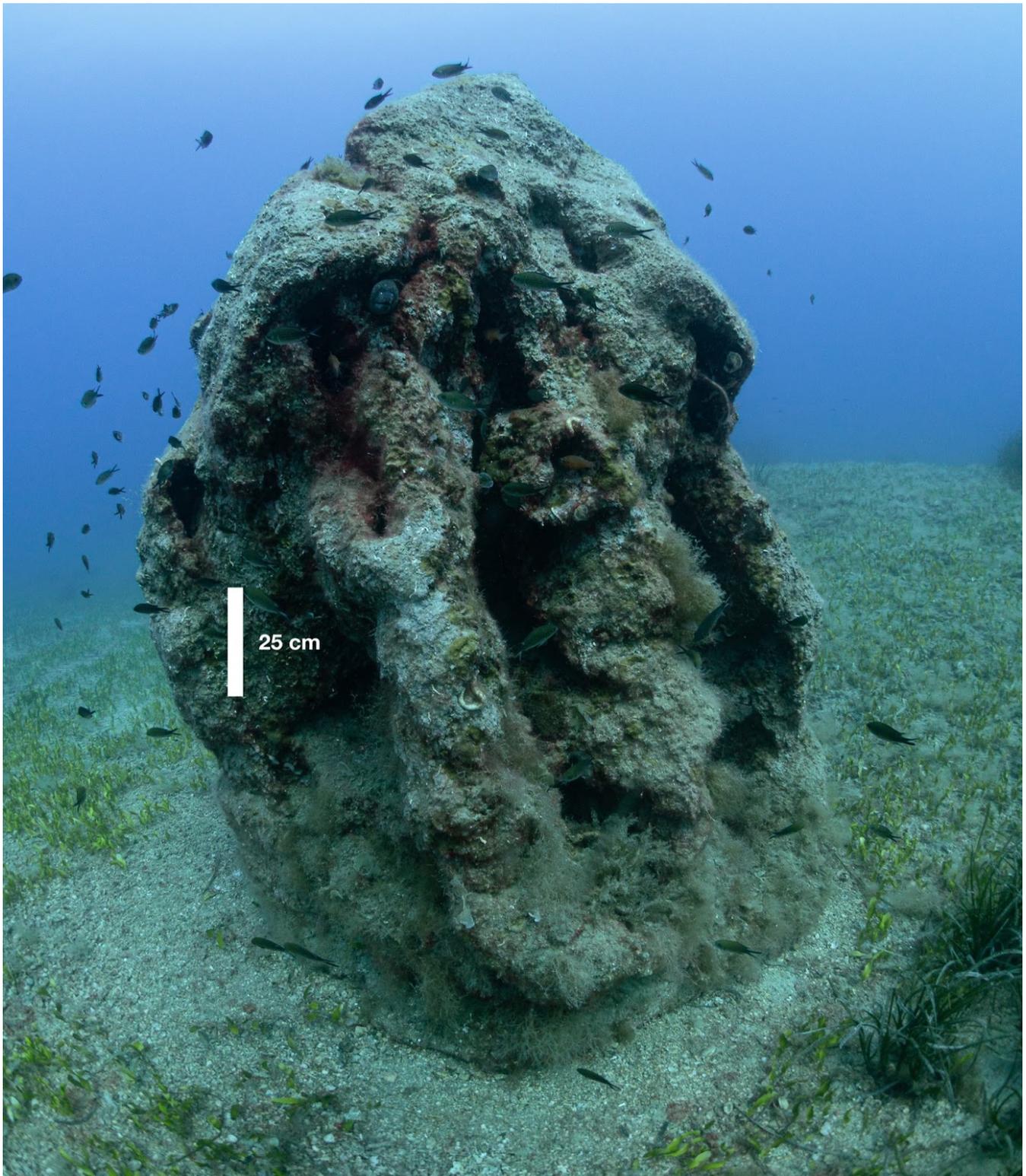
### 2.2. Sampling and Image Processing

Sampling was implemented with scuba diving in 2017 (June), 2019 (October), and 2020 (October). Seawater temperatures for the months of sampling were 23 °C for June 2017 and 25 °C for October 2019 and 2020. For the study of sessile benthos, five ARs, randomly selected in each period, were sampled with 25 × 25 cm photoquadrats (Figure S1). Five replicate quadrats per sampling event were randomly positioned over flat, exposed areas of each AR and photographed, resulting in a total of 75 photos (25 in each sampling

event). For this purpose, a digital mirrorless camera (Olympus OM-D E-M5) was used, paired with two external underwater strobes to ensure consistent illumination. The analysis of photoquadrats is a non-destructive and cost-efficient sampling method that is widely used for the studying and monitoring of sessile communities in both natural and artificial substrates [16–18,21]. Additionally, qualitative samples of several taxa were collected for taxonomic identification in the lab or photographed *in situ*.



**Figure 1.** (a) Map of the Eastern Mediterranean Sea. (b) Location of the Underwater Biotechnological Park of Crete (UBPC) (red arrow), almost 2 km offshore from the northern coast of Crete in Greece.



**Figure 2.** Concrete artificial reef installed at the Underwater Biotechnological Park of Crete (UBPC), photographed 4 years post-deployment (Photo credit: Thanos Dailianis).

The cover percentages of taxonomic categories from photoquadrat data were calculated with an advanced image analysis using PhotoQuad [23] which is a free software specialized for underwater ecological applications in integrating various methods and tools for the accurate calculation of species coverage. In each photo sample, 100 random points

were spawned within the photo frame. The points over living benthic species were assigned to a specific taxon or morpho-functional group (e.g., Mucilaginous algae or Encrusting Bryozoa). Every single point falling on a biogenic substrate that was impossible to be identified through visual census was designated as an “Unidentified Biogenic Substrate” (UBS) [17,24]. UBS describes the substrates covered by small-sized organisms (e.g., bacterial colonies, padded algae in their early stages of development, encrusting assemblages with no clear characteristics, etc.). To a lesser extent, UBS may refer to blurry and shady areas of biogenic cover in the photo samples, due to substrate rugosity.

For the study of motile associated fauna, six replicate padded quadrats of 25 × 25 cm were randomly exhaustively scraped and collected from three random ARs each year with a MANOSS suction sampler (Figure S2) [20]. The motile macrofauna taxa were collected after sieving with a mesh size of 0.5 mm; preserved in 97% ethanol; sorted; and identified to the family/species level for Polychaeta, Crustacea, and Mollusca. Preserved samples were deposited in the collection of the Biodiversity Laboratory of IMBBC-HCMR.

### 2.3. Statistical Analysis

Statistical analysis was performed on species abundances with the PRIMER (ver. 6) software package [25]. To mitigate the contribution of the most abundant species, data were transformed under the fourth root formula, and a triangular similarity matrix was created based on the Bray–Curtis similarity index [26]. In order to investigate spatial patterns in the community structure, a non-metric multidimensional scaling (MDS) was performed separately for the sessile and motile communities. To identify the taxa that contributed most to the multivariate patterns of the sessile and motile communities each year, the similarity percentages (SIMPER) analysis was used. The significance of the multivariate results was assessed using a one-way analysis of similarity (ANOSIM) test on the transformed data. The null hypothesis of the ANOSIM test was that there are no differences in biodiversity patterns among the sampling periods.

## 3. Results

### 3.1. Community Structure

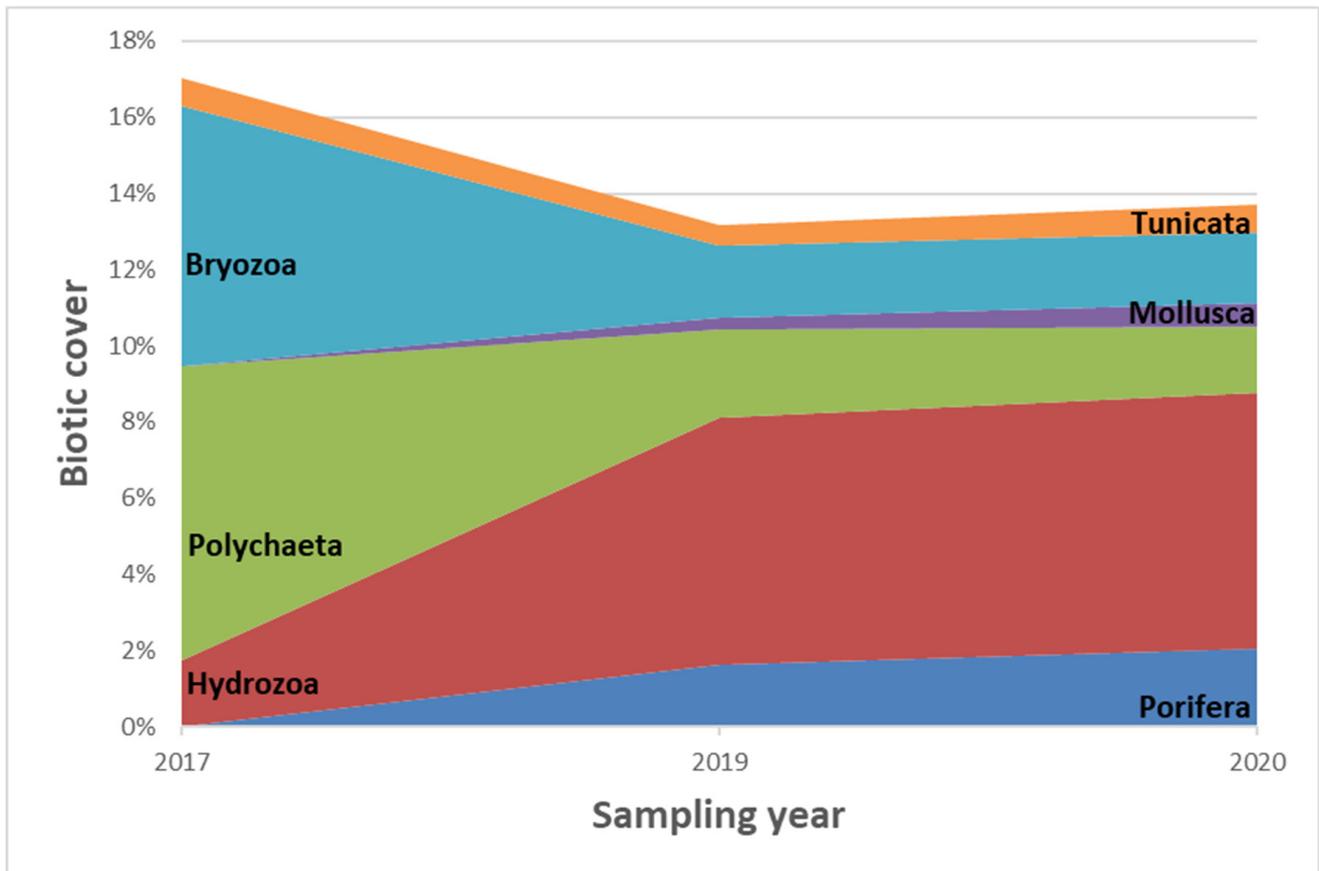
#### 3.1.1. Sessile Community

A total of 37 sessile taxa were identified in the 75 analyzed photoquadrats, belonging to six major taxonomic groups (12 Macroalgae, 8 Porifera, 1 Hydrozoa, 3 Polychaeta, 6 Mollusca, 3 Bryozoa, and 4 Tunicata).

Macroalgae dominated in terms of the surface cover consistently over the studied period, with 82.3% coverage in 2017, 86.8% in 2019, and 86.3% in 2020 (Table S1). In the earliest year after deployment (2017), the turf-forming algae coverage (37.0%) was much lower than the other sampling periods (80.6% in 2019 and 73.7% in 2020), while Mucilaginous algae (27.8%) and *Dictyota implexa* (13.3%), which covered a significant amount of the surface, were completely absent in the following years. The encrusting macroalgae comprised mostly *Peyssonnelia* species, while the turf-forming algae were characterized mainly by Rhodophyta such as *Jania* sp. and *Laurencia* sp.

Sessile invertebrates contributed with 17.0% coverage in 2017, which was considered the initial stage of colonization. Polychaeta and Bryozoa dominated (7.7% and 6.9%, respectively) (Figure 3 and Table S1), with Serpulidae being the most abundant polychaete family. Thereafter, Cnidaria, represented only by Hydrozoa (1.7%), and Tunicata (0.7%), followed. Porifera and Mollusca were completely absent from the photoquadrats of 2017. Interestingly, in the following years (2019 and 2020), the sessile invertebrate coverage decreased (13.2% and 13.7%, respectively). Polychaeta (2.3% and 1.7%) and Bryozoa (1.9% and 1.9%) showed a decreasing trend in favor of Cnidaria (6.5% and 6.7%). Porifera (1.6% and 2.1%) and Mollusca (0.3% and 0.6%) showed a slight increasing trend, while Tunicata (0.5% and 0.8%) remained stable throughout the sampling periods. A considerable percentage of the AR surface was classified as UBS, as it represented a biogenic substrate that could not be safely assigned to any specific taxonomic category (19.7% in 2017, 47.6%

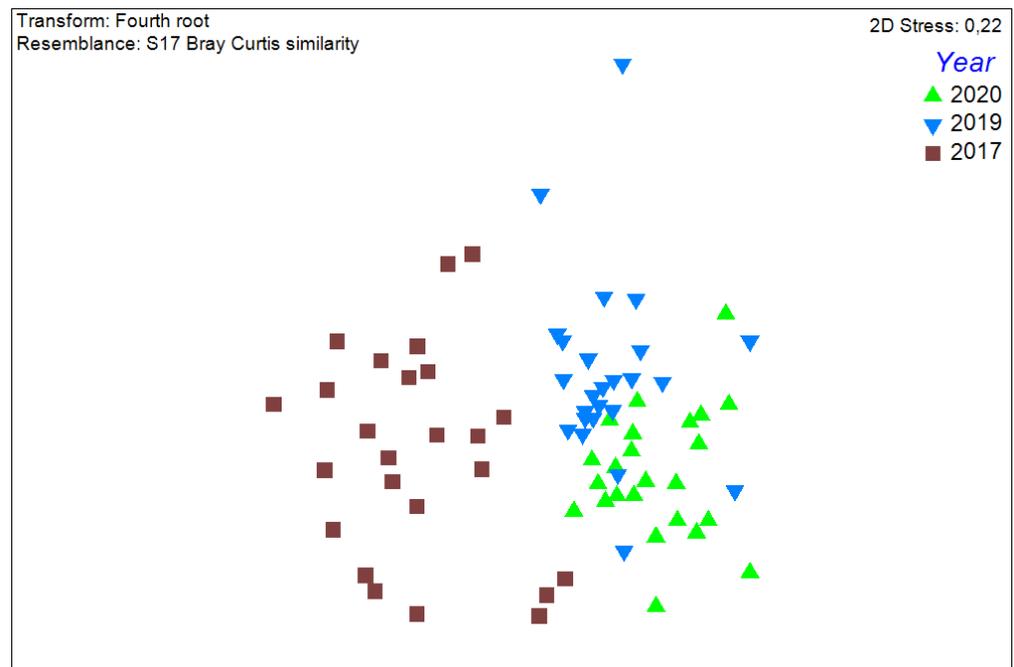
in 2019, and 43.6% in 2020). This category was excluded from the statistical analysis, as it could not be assigned to a more specific morpho-functional group. In all sampling periods, the substrate was almost completely covered by sessile benthos (mean coverage: 95.0% in 2017, 97.6% in 2019, and 99.9% in 2020).



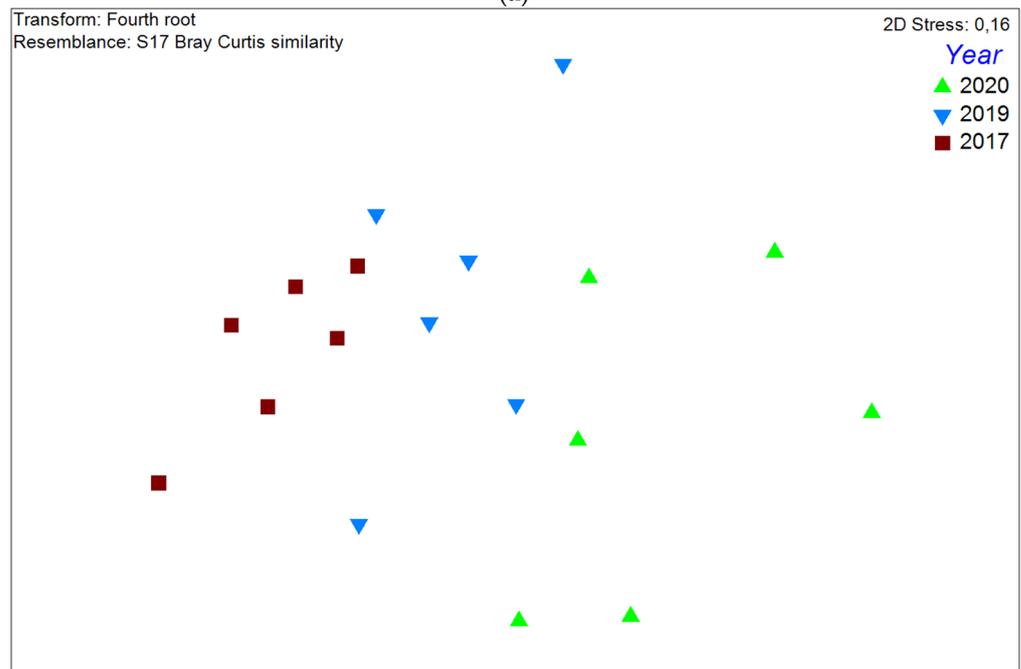
**Figure 3.** Biotic mean coverage of the major taxonomic groups of sessile invertebrates (%) showing changes over the years. Colors indicate different major taxonomic groups that contributed to the coverage of photoquadrats of the artificial reefs.

Additionally, three non-indigenous species (NIS) have been reported in photoquadrats: the brown alga *Styopodium schimperi* with increasing coverage from 0.5% in 2017 to 1.3% in 2020 and two NIS of Mollusca, *Pinctada radiata* with 0.4% in 2019 and *Dendostrea cf. folium* in 2020 with 0.1%.

The MDS analysis revealed a clear differentiation among the three sampling periods regarding sessile communities (Figure 4a). The community of 2017 tended to be more distinctive from subsequent years 2019 and 2020, forming a well-defined cluster. One-way ANOSIM showed a discrimination among the three groups ( $R = 0.443$ ,  $p < 0.01$ ). Additional pairwise tests revealed significant differences between 2017 and 2019 ( $R = 0.516$ ,  $p < 0.01$ ), between 2017 and 2020 ( $R = 0.685$ ,  $p < 0.01$ ), and between 2019 and 2020 ( $R = 0.167$ ,  $p < 0.01$ ), supporting the MDS results.



(a)



(b)

**Figure 4.** Non-metric multidimensional scaling (MDS) plots showing the similarities among the three sampling periods (indicated by different colors) between: (a) sessile communities and (b) motile communities.

A SIMPER analysis between 2017 and the other two years showed that the Bray–Curtis dissimilarity was mainly due to Mucilaginous algae showing 52.37% dissimilarity with 2019 and 54.71% with 2020 (Table 1). Between 2019 and 2020, the dissimilarity was much lower (39.20%), with encrusting macroalgae contributing the most. The similarity of each sampling period and the taxa that contributed most to the fauna patterns are presented in Table 2. The category of turf-forming algae contributed the most to the similarity of each year, followed by Cnidaria (Hydrozoa) in 2019 and 2020 and, in 2017, by Mucilaginous

algae, which disappeared the following years. The similarity index increased over the years (56.12% in 2017, 63.99% in 2019, and 64.47% in 2020).

**Table 1.** Pairwise similarity percentage analysis (SIMPER), showing the pairwise average dissimilarity (%) among three sampling periods.

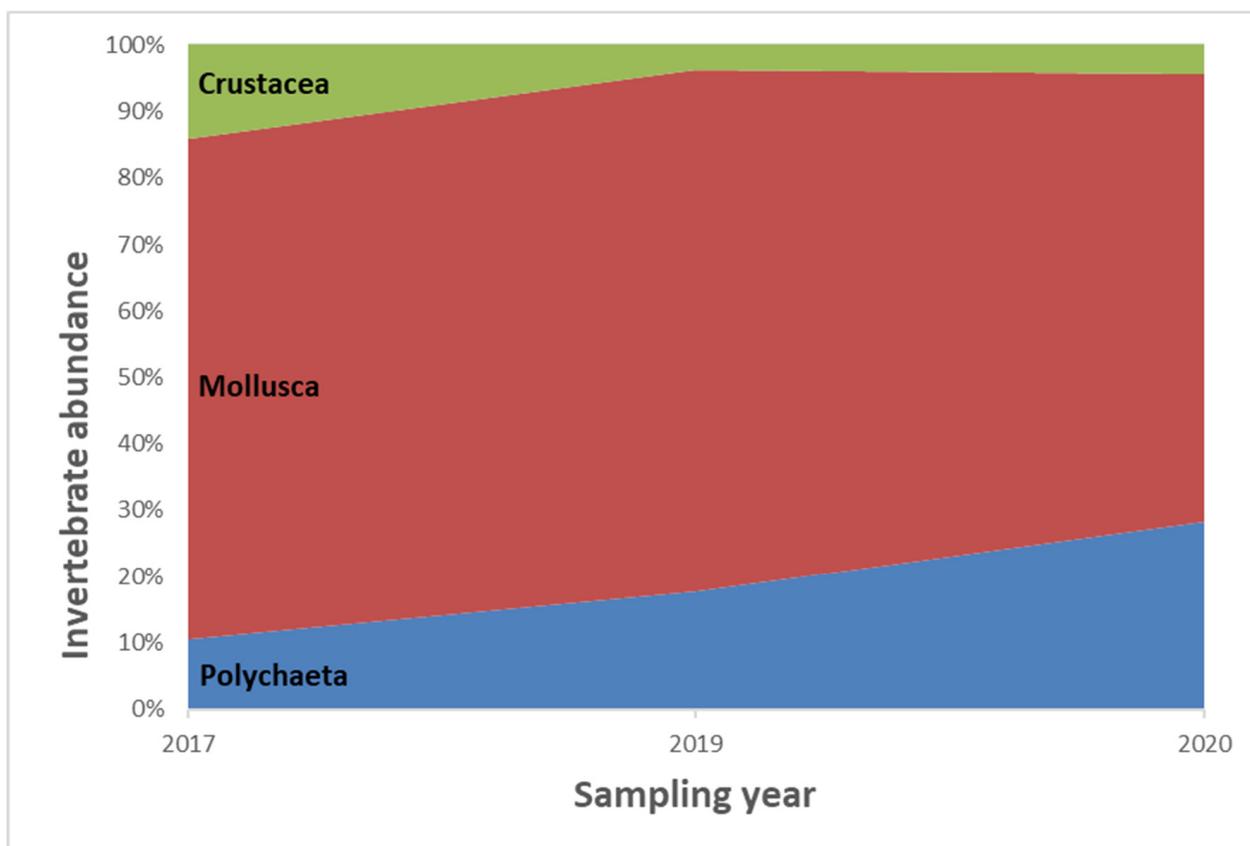
Sessile Assemblages		Motile Assemblages	
Sampling Periods	Average Dissimilarity	Sampling Periods	Average Dissimilarity
2017–2019	52.37	2017–2019	61.52
2017–2020	54.71	2017–2020	74.45
2019–2020	39.2	2019–2020	67.83

**Table 2.** Similarity percentage analysis (SIMPER), showing the contribution of common taxa to the average similarity (%) in each year of sampling on the artificial reefs.

Major Taxonomic Group		2017	2019	2020
<b>Sessile Assemblages</b>		<b>56.12</b>	<b>63.99</b>	<b>64.47</b>
	<b>Average Similarity</b>			
	Taxonomic Group/Species			
Macroalgae	Turf-forming algae	34.43	49.25	39.23
	Encrusting macroalgae	7.31	12.92	16.46
	Mucilaginous algae	19.82	-	-
	<i>Acetabularia</i> sp.	5.56	-	-
	<i>Dictyota implexa</i>	3.87	-	-
Cnidaria	Hydrozoa	4.97	16.92	17.36
Polychaeta	Other Serpulidae	17.77	16.90	12.71
	<i>Protula</i> spp.	-	-	5.66
<b>Motile assemblages</b>		<b>53.87</b>	<b>40.58</b>	<b>33.77</b>
	<b>Average Similarity</b>			
	Family			
Polychaeta	Capitellidae	-	6.42	-
	Chrysopetalidae	6.22	13.74	7.78
	Dorvilleidae	-	4.13	-
	Eunicidae	3.28	-	-
	Nereididae	3.39	-	2.57
	Paraonidae	4.06	-	-
	Syllidae	4.13	8.65	-
Mollusca	Cerithiidae	24.92	36.59	73.86
	Rissoidae	7.78	9.19	-
	Trochidae	-	-	-
Crustacea	Aoridae	9.41	7.89	-
	Bodotriidae	4.20	-	-
	Caprellidae	7.78	-	-
	Dexaminidae	10.50	-	-
	Lysianassidae	6.09	-	-
	Pasiphaeidae	-	4.24	7.14

### 3.1.2. Motile Community

Overall, 1898 individual macroinvertebrates (1130 in 2017, 583 in 2019, and 185 in 2020) were counted and identified at least to the family level in 18 scraped quadrats. These were classified as Polychaeta (14.4%), Mollusca (75.5%), and Crustacea (10.0%). The mean contribution of each taxon is presented in Figure 5 and Table S2. The participation of Mollusca was the highest during all the sampling periods, represented by 33 species of 19 families. *Bittium latreillii* was the most abundant species in all the samples, with a contribution of 63.2–69.1%. Polychaeta were represented by 15 families and showed an increase in their contribution over the years (10.5% in 2017, 17.6% in 2019, and 28.1% in 2020), while Crustacea, represented by 16 families, decreased from 14.2% in 2017 to 3.6 and 4.3 in 2019 and 2020, respectively.



**Figure 5.** Relative abundance (%) of the major motile invertebrate taxonomic groups per year. Colors indicate different major taxonomic groups of the invertebrates identified from the scrapes of the MANOSS sampler.

The MDS analysis showed a clear distinction between the communities of 2017 and 2020, while 2019 stood as a discrete cluster between the two other sampling years (Figure 4b). Additionally, one-way ANOSIM showed a discrimination among the sampling periods ( $R = 0.417$ ,  $p < 0.01$ ). Additional pairwise tests revealed significant differences between 2017 and 2019 ( $R = 0.389$ ,  $p < 0.01$ ), 2017 and 2020 ( $R = 0.717$ ,  $p < 0.01$ ), and 2019 and 2020 ( $R = 0.217$ ,  $p < 0.01$ ). It should be noted that the motile community of 2017 showed a more accumulated distribution, contrary to the pattern of depiction from the sessile data.

The SIMPER analysis on the motile community showed great dissimilarity among the sampling periods compared to the sessile SIMPER results (Table 1). The Bray–Curtis dissimilarity index between 2017 and 2020 was 74.45%, with Dexaminidae (Crustacea) contributing the most. The dissimilarity percentages between 2019 and 2020 were 67.83%, with Syllidae (Polychaeta) contributing the most, and between 2017 and 2019 was 61.52%, with Caprellidae (Crustacea) contributing the most. The similarity of each sampling year and the families that contributed most to the similarity pattern are presented in Table 2. Specifically, the family Cerithidea (Mollusca) was the main driver for the assessed dissimilarity between the sampling years due to the high abundance of the species *B. latreillii*. The result of the similarity index complied with the findings of the MDS analysis, showing a much higher similarity within the 2017 replicates (53.87%) than the other two periods (40.58% in 2019 and 33.77% in 2020). They were, however, in contrast with the patterns observed for the sessile community analysis.

#### 4. Discussion

Significant shifts in the structure of benthic assemblages on the ARs were observed during the three sampling periods over a five-year survey following the initial deployment.

This can be attributed to the community transformation of the early successional stages of the benthic assemblages into an oligotrophic environment such as the Southeastern Mediterranean before reaching a climax community structure [27–29].

Two years after their deployment, the ARs were mainly covered by macroalgae (78%) divided into the three morpho-functional categories: (a) “turf-forming algae”, (b) the canopy-forming brown alga *D. implexa*, and (c) opportunistic filamentous Mucilaginous algae. At the same time, the recorded abundance of the motile species was the largest compared to the subsequent samplings. Similar results were reported in the past for newly deployed concrete ARs located in an oligotrophic environment on a sandy seabed with *P. oceanica* seagrass meadows [30,31]. In particular, D’Anna et al. [31] identified the opportunistic alga as *Lophocladia lallemandii*, which is visually similar to the corresponding alga in this study. The presence of seasonal algae on the ARs is probably related to shifting temperature conditions resulting from the different sampling months (June for 2017, as opposed to October for 2019 and 2020). Additionally, the presence of this taxon is probably responsible for the lower cover of UBS in 2017. These differences could also be partly responsible for the pronounced succession patterns observed for the sessile communities between 2017 and 2019. A series of seasonal sampling campaigns within the year could provide important information on this phenomenon in the future. Turf-forming algae are characterized by their rapid expansion on a broad spatial scale, while they are not significantly affected by environmental disturbances [32–34]. Therefore, this category seems to prevail in hard sublittoral substrates, especially in artificial ones [35–37]. Four years post-deployment, the turf-forming algae increased their size and total coverage, effectively displacing the other two species completely. Encrusting macroalgae, which mainly comprised *Peyssonnelia* species, were first recorded two years after deployment and gradually increased in size and abundance in the following years, in contrast to D’Anna et al. [31], who reported that colonization of *Peyssonnelia* sp. started four years after deployment. A detailed taxonomic description of the algae species in the ARs of UPBC most probably will further clarify the trends in the present study.

The coverage of sessile invertebrates (15–19%) was much lower compared to the macroalgae. This pattern is very common in early and middle successional stages on hard substrates [38,39]. Sessile Polychaeta and Bryozoa were the main faunistic colonizers two years after the deployment of the ARs. Serpulids, which thrived in 2017, showed a decreasing trend the following years, as also reported by Casoli et al. [40]. Yet, this can probably be explained by the increase in size of the macroalgae, which may be concealing the smaller-sized Polychaeta underneath. Serpulids have similarly been reported in high abundance in eutrophic [1,41] and oligotrophic areas [29,34,42]. Erect Bryozoans are relatively rare in exposed sublittoral substrates of the Eastern Mediterranean compared to the Western Basin [19], while encrusting Bryozoans are usually fast colonizers of hard substrates in the early succession stages [34,42,43]. In the present study, the coverage of Bryozoa during the first assessment period was in line with the above observations, while the only erect Bryozoan record was *Reteporella* sp. with a low presence. Hydrozoa, which are abundant in most benthic communities of the Mediterranean Sea [44], were found to increase their coverage in this study over the years. This upward trend has also been recorded in previous studies [45–47] and correlated with the gradual maturation of the sessile assemblages [47]. Porifera and Mollusca were completely absent from the first assessment in 2017 but gradually colonized the ARs and tended to gain ground over time (also in Casoli et al. [40]), while the presence of Tunicata was very rare. Compared to other studies, Porifera and Tunicata were scarcely observed [45,48], but this trend conformed to Antoniadou et al. [34], who reported a similar trend in an oligotrophic Mediterranean environment. The absence of slow-growing demosponges with a massive growth form, typical for Mediterranean waters, can be attributed to the early phase of the colonization progress. Generally, pronounced ecological changes on sublittoral ARs usually occur during secondary succession [49], which is a gradual process in time, driving the shift towards the climax community [50]. However, many biological communities remain in a continual state

of secondary succession, especially when random disturbances persist [51]. The increasing presence of NIS (*S. schimperi*, *P. radiata*, and *D. cf. folium*) in the studied ARs across the sampling events are related to the dynamics of the marine invasions prevalent in Eastern Mediterranean marine ecosystems [52]. The large coverage of UBS is linked to the standard limitations in the identification of small-sized biota based only on visual methods [17,24] and, especially, thin biogenic mats during the early succession stages. Future analyses using metabarcoding could elucidate the nature of UBS on artificial substrates by either identifying the preliminary states of settlement of the benthic assemblages [53] or identifying bacterial biofilms that are essential for the subsequent invertebrate larval settlement [54]. In general, very few data exist considering the succession of motile assemblages on sublittoral hard substrates [42], since most relevant studies usually focus on sessile species [55,56].

Regarding motile fauna, Mollusca were the dominant phylum, and this is mainly due to the very high abundance of *B. latreillii* (>60%). This high abundance of *B. latreillii* has been reported on hard substrates of the Eastern Mediterranean in the past and has been attributed to the high presence of turf-forming algae, which traps organic matter, providing suitable living conditions for this gastropod species [57–59]. Additionally, it displays rapid growth during warm periods and high abundance in *P. oceanica* seagrass meadows at 15–25 m depths, where its recruitment takes place [60].

Polychaetes are one of the most abundant invertebrate groups in Mediterranean ecosystems [61–63]. Motile Polychaeta showed an increasing trend regarding their abundance over the studied timespan. This result contradicts the corresponding trend observed for sessile Polychaeta. Previous studies on Mediterranean ARs have shown both decreasing [7] and increasing trends [34,42] for motile Polychaeta abundance. The abundance of Crustacea has been substantially reduced since 2017 from about 14% to ca. 4%. The high rates of Crustacea in 2017 are probably linked to the strong presence of the mucilaginous algae that create a favorable environment for this taxon by increasing the biomass and available growth space [64].

Overall, through the sampling years, a decreasing trend was observed for motile invertebrate abundances, while the diversity seems to be consistent or slightly decreasing. Changes in motile abundances could be affected—at least to a certain point—by bias in the sampling method (i.e., the padded quadrat frames require a relatively flat surface in order to eliminate the escape of high mobility specimens during the scraping phase). In addition, stochasticity due to prevailing environmental conditions, such as strong currents and wave actions, may drive motile species to more protected places such as cavities or under algal canopies, and the nearby seagrass meadow, because smooth surfaces are exposed more to hydrodynamism. The overall effectiveness of the MANOSS suction sampler has been previously evaluated for similar habitats in comparison with other standard hard-bottom sampling methods [65], showing no significant differences in the sampling efficiency but an increased performance in the number of species collected and efficiency in the retention of small-sized taxa (i.e., meiofauna).

The presence of mucilaginous algae at the first sampling event increased the complexity and the algal biomass of the scraped samples, thus resulting in high abundances of benthic fauna, as has previously been reported for similar systems [31,42,64]. Non-parametric community analyses showed that only two families of motile invertebrates were shared in all three sampling periods (i.e., Cerithiidae and Chrysopetalidae). Contrastingly, four taxa contributed most to the similarity patterns of the sessile community (i.e., turf-forming algae, encrusting macroalgae, Hydrozoa, and Serpulidae), resulting in a more stable structure compared to that of the motile cluster. The similarity between the replicate samples (within the sampling year) seemed to be reduced over the sampling years (as expressed by distances in the MDS plot of Figure 4b). On the other hand, no clear pattern can be revealed from motile fauna, reflecting the differential responses of motile and sessile invertebrates to the deployment of ARs.

Our results clearly confirm that artificial structures are subject to highly complex colonization patterns. This is especially true for the oligotrophic Eastern Mediterranean

environment, where a lack of abundance of trophic sources hinders the development of fast-growing organisms with substantial body sizes [66,67]. Extended monitoring periods (>5 years) can help elucidate the processes that shape typical hard substrate communities, moving gradually from the initial stages of a settlement to more mature community forms. Different assessment methods (visual and scraped quadrats) can be complementary to this end, since each one reveals distinct clusters of the overall assemblage, and reliance on just one may produce partial results. It would be interesting for future studies to assess the changes in similar local natural habitats, especially in the face of a rapidly changing Mediterranean environment [68], to evaluate the extent to which a community shift, as reported in our study, reflects succession patterns on artificial surfaces or global change patterns occurring at the ecosystem level.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/jmse10050620/s1>: Table S1. Mean coverage percentage  $\pm$  standard deviation of sessile taxa on artificial reefs per year. Table S2. Mean abundance percentage  $\pm$  standard deviation of motile invertebrates on artificial reefs per year. Figure S1. Photographed quadrat of 25  $\times$  25 cm placed on the surface of an AR. Figure S2. Sampling of the motile component of benthic assemblages.

**Author Contributions:** Scientific supervision, T.D. and V.G.; sample collection, T.D., G.C., E.V. and V.G.; laboratory analysis, M.F., F.S., A.P., M.C.-B., E.V. and W.P.; data analysis, I.R., G.C., T.D. and V.G.; writing—original draft preparation, I.R.; writing—review and editing, G.C., V.G., T.D., C.D. and P.K.; and funding acquisition, C.D. and P.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded in the context of the projects: (a) “Centre for the study and sustainable exploitation of Marine Biological Resources (CMBR)” (MIS 5002670), which was implemented under the Action “Reinforcement of the Research and Innovation Infrastructure”, funded by the Operational Programme “Competitiveness, Entrepreneurship and Innovation” (NSRF 2014–2020) and co-financed by Greece and the European Union (European Regional Development Fund), and (b) “Recreational Diving Oasis with Artificial Reefs” (MIS 5027402) by the Regional Operational Programme (ROP) Crete 2014–2020, Operational Programme “Competitiveness, Entrepreneurship and Innovation” (NSRF 2014–2020), co-financed by Greece and the European Union (European Regional Development Fund—ERDF).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data is contained within the article or supplementary material.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Manoudis, G.; Antoniadou, C.; Dounas, K.; Chintiroglou, C. Successional stages of experimental artificial reefs deployed in vistonikos gulf (N. Aegean Sea, Greece): Preliminary results. *Belg. J. Zool.* **2005**, *135*, 209–215.
2. Airoidi, L.; Beck, M.W. Loss, status and trends for coastal marine habitats of Europe. *Oceanogr. Mar. Biol.* **2007**, *45*, 345–405. [[CrossRef](#)]
3. Fabi, G.; Spagnolo, A.; Bellan-Santini, D.; Charbonnel, E.; Çiçek, B.A.; García, J.J.G.; Jensen, A.C.; Kallianiotis, A. Overview on artificial reefs in Europe. *Braz. J. Oceanogr.* **2011**, *59*, 155–166. [[CrossRef](#)]
4. Sinis, A.I.; Chintiroglou, C.C.; Stergiou, K.I. Preliminary results from the establishment of experimental artificial reefs in the N. Aegean Sea (Chalkidiki, Greece). *Belg. J. Zool.* **2000**, *130*, 143–147.
5. Klaoudatos, D.S.; Anastasopoulou, A.; Papaconstantinou, C.; Conides, A.I. The Greek experience of artificial reef construction and management. *J. Environ. Prot. Ecol.* **2012**, *13*, 1647–1655.
6. Seaman, W. Unifying trends and opportunities in global artificial reef research, including evaluation. *ICES J. Mar. Sci.* **2002**, *59*, S14–S16. [[CrossRef](#)]
7. Achilleos, K.; Patsalidou, M.; Jimenez, C.; Kamidis, N.; Georgiou, A.; Petrou, A.; Kallianiotis, A. Epibenthic communities on artificial reefs in Greece, Mediterranean Sea. *Water* **2018**, *10*, 347. [[CrossRef](#)]
8. Psarra, S.; Tselepidis, A.; Ignatiades, L. Primary productivity in the Oligotrophic Cretan Sea (NE Mediterranean): Seasonal and interannual variability. *Prog. Oceanogr.* **2000**, *46*, 187–204. [[CrossRef](#)]

9. Tselepides, A.; Papadopoulou, K.N.; Podaras, D.; Plaiti, W.; Koutsoubas, D. Macrobenthic Community Structure over the continental margin of Crete (South Aegean Sea, NE Mediterranean). *Prog. Oceanogr.* **2000**, *46*, 401–428. [[CrossRef](#)]
10. Georgopoulos, D.; Theocharis, A.; Zodiatis, G. Intermediate water formation in the Cretan Sea (South Aegean Sea). *Oceanol. Acta* **1989**, *12*, 353–359.
11. Androulakis, D.N.; Dounas, C.G.; Margaris, D.P. The seafloor observatory of the underwater biotechnological park of Crete. In Proceedings of the 6th International Conference on “Experiments/Process/System Modelling/Simulation/Optimization”, Athens, Greece, 8–11 July 2015.
12. Dounas, C.; Androulakis, D.; Dailianis, A.; Koulouri, P. Recreational diving Oasis with artificial habitats. *Rapp. Comm. Int. Mer Médit* **2016**, *41*, 476.
13. Jensen, A.; Collins, K.; Lockwood, P. Current issues relating to artificial reefs in European seas. In *Artificial Reefs in European Seas*; Jensen, A.C., Collins, K.J., Lockwood, A.P.M., Eds.; Springer: Dordrecht, The Netherlands, 2000; pp. 489–499. ISBN 978-0-7923-6144-2.
14. Jimenez, C.; Hadjioannou, L.; Petrou, A.; Andreou, V.; Georgiou, A. Fouling communities of two accidental artificial reefs (modern shipwrecks) in Cyprus (Levantine sea). *Water* **2016**, *9*, 11. [[CrossRef](#)]
15. Jimenez, C.; Andreou, V.; Evriviadou, M.; Munkes, B.; Hadjioannou, L.; Petrou, A.; Abu Alhaja, R. Epibenthic communities associated with unintentional artificial reefs (modern shipwrecks) under contrasting regimes of nutrients in the Levantine sea (Cyprus and Lebanon). *PLoS ONE* **2017**, *12*, e0182486. [[CrossRef](#)] [[PubMed](#)]
16. Gerovasileiou, V.; Voultziadou, E. Sponge diversity gradients in Marine caves of the Eastern Mediterranean. *J. Mar. Biol. Assoc. UK* **2016**, *96*, 407–416. [[CrossRef](#)]
17. Dimarchopoulou, D.; Gerovasileiou, V.; Voultziadou, E. Spatial variability of sessile benthos in a semi-submerged Marine cave of a remote Aegean Island (Eastern Mediterranean sea). *Reg. Stud. Mar. Sci.* **2018**, *17*, 102–111. [[CrossRef](#)]
18. Sedano, F.; Florido, M.; Rallis, I.; Espinosa, F.; Gerovasileiou, V. Comparing sessile benthos on shallow artificial versus natural hard substrates in the Eastern Mediterranean sea. *Mediterr. Mar. Sci.* **2019**, *20*, 688. [[CrossRef](#)]
19. Çinar, M.E.; Féral, J.; Arvanitidis, C.; David, R.; Taşkın, E.; Sini, M.; Dailianis, T.; Doğan, A.; Gerovasileiou, V.; Evcen, A.; et al. Coralligenous assemblages along their geographical distribution: Testing of concepts and implications for management. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2020**, *30*, 1578–1594. [[CrossRef](#)]
20. Chatzigeorgiou, G.; Faulwetter, S.; López, E.; Sardá, R.; Arvanitidis, C. Can coastal biodiversity measured in four Mediterranean sites be representative of the region? A test for the robustness of the NaGISA protocol by using the hard substrate syllid (Annelida, Polychaeta) taxo-communities as a Surrogate. *Hydrobiologia* **2012**, *691*, 147–156. [[CrossRef](#)]
21. Bianchi, C.N.; Pronzato, R.; Cattaneo-Vietti, R.; Benedetti Cecchi, L.; Morri, C.; Pansini, M.; Chemello, R.; Milazzo, M.; Frascchetti, S.; Terlizzi, A.; et al. Hard bottoms. In *Mediterranean Marine Benthos: A Manual of Methods for Its Sampling and Study*; Gambi, M.C., Dappiano, M., Eds.; Società Italiana di Biologia Marina: Genova, Italy, 2004; Volume 11, (Suppl. S1), pp. 185–215.
22. Androulakis, D.; Dounas, C.; Banks, A.; Magoulas, A.; Margaris, D. An assessment of computational fluid dynamics as a tool to aid the design of the HCMR-artificial-reefs<sup>TM</sup> diving Oasis in the underwater biotechnological park of Crete. *Sustainability* **2020**, *12*, 4847. [[CrossRef](#)]
23. Trygonis, V.; Sini, M. PhotoQuad: A dedicated seabed image processing software, and a comparative error analysis of four photoquadrat methods. *J. Exp. Mar. Biol. Ecol.* **2012**, *424–425*, 99–108. [[CrossRef](#)]
24. Gerovasileiou, V.; Dimitriadis, C.; Arvanitidis, C.; Voultziadou, E. Taxonomic and functional surrogates of sessile benthic diversity in Mediterranean Marine caves. *PLoS ONE* **2017**, *12*, e0183707. [[CrossRef](#)] [[PubMed](#)]
25. Clarke, K.R.; Gorley, R.N. *PRIMER v6: User Manual/Tutorial*; PRIMER-E Ltd.: Plymouth, UK, 2006.
26. Clarke, K.; Warwick, R. A further biodiversity index applicable to species lists: Variation in taxonomic distinctness. *Mar. Ecol. Prog. Ser.* **2001**, *216*, 265–278. [[CrossRef](#)]
27. Ardizzone, G.; Somaschini, A.; Belluscio, A. Prediction of benthic and fish colonization on the fregene and other Mediterranean artificial reefs. In *Artificial Reefs in European Seas*; Jensen, A.C., Collins, K.J., Lockwood, A.P.M., Eds.; Springer: Dordrecht, The Netherlands, 2000; pp. 113–128. ISBN 978-0-7923-6144-2.
28. Riggio, S.; Badalamenti, F.; D’Anna, G. Artificial reefs in Sicily: An overview. In *Artificial Reefs in European Seas*; Jensen, A.C., Collins, K.J., Lockwood, A.P.M., Eds.; Springer: Dordrecht, The Netherlands, 2000; pp. 65–74. ISBN 978-0-7923-6144-2.
29. Kocak, F.; Ergen, Z.; Çinar, M.E. Fouling organisms and their developments in a polluted and an unpolluted marina in the Aegean sea (Turkey). *Ophelia* **1999**, *50*, 1–20. [[CrossRef](#)]
30. Badalamenti, F.; D’Anna, G.; Riggio, S. Artificial reefs in the gulf of castellammare (North-West Sicily): A case study. In *Artificial Reefs in European Seas*; Jensen, A.C., Collins, K.J., Lockwood, A.P.M., Eds.; Springer: Dordrecht, The Netherlands, 2000; pp. 75–96. ISBN 978-0-7923-6144-2.
31. D’Anna, G.; Badalamenti, F.; Riggio, S. Artificial reefs in North West Sicily: Comparisons and conclusions. In *Artificial Reefs in European Seas*; Jensen, A.C., Collins, K.J., Lockwood, A.P.M., Eds.; Springer: Dordrecht, The Netherlands, 2000; pp. 97–112. ISBN 978-0-7923-6144-2.
32. Rindi, F.; Cinelli, F. Phenology and Small-scale distribution of some rhodomelacean red algae on a Western Mediterranean Rocky Shore. *Eur. J. Phycol.* **2000**, *35*, 115–125. [[CrossRef](#)]

33. Airoidi, L. Effects of disturbance, life histories, and overgrowth on coexistence of algal crusts and turfs. *Ecology* **2000**, *81*, 798–814. [[CrossRef](#)]
34. Antoniadou, C.; Voultziadou, E.; Chintiroglou, C. Seasonal patterns of colonization and early succession on sublittoral rocky cliffs. *J. Exp. Mar. Biol. Ecol.* **2011**, *403*, 21–30. [[CrossRef](#)]
35. Falace, A. Evaluation of the influence of inclination of substrate panels on seasonal changes in a macrophytobenthic community. *ICES J. Mar. Sci.* **2002**, *59*, S116–S121. [[CrossRef](#)]
36. Burt, J.; Bartholomew, A.; Sale, P.F. Benthic development on large-scale engineered reefs: A comparison of communities among breakwaters of different age and natural reefs. *Ecol. Eng.* **2011**, *37*, 191–198. [[CrossRef](#)]
37. Tsiamis, K.; Salomidi, M.; Gerakaris, V.; Mogg, A.O.M.; Porter, E.S.; Sayer, M.D.J.; Küpper, F.C. Macroalgal vegetation on a North European artificial reef (Loch Linnhe, Scotland): Biodiversity, community types and role of abiotic factors. *J. Appl. Phycol.* **2020**, *32*, 1353–1363. [[CrossRef](#)]
38. Bailey-Brock, J.H. Fouling community development on an artificial reef in Hawaiian waters. *Bull. Mar. Sci.* **1989**, *42*, 580–591.
39. Palmer-Zwahlen, M.L.; Asetline, D.A. Successional development of the turf community on a Quarry rock artificial reef. *Bull. Mar. Sci.* **1994**, *55*, 902–923.
40. Casoli, E.; Ricci, S.; Antonelli, F.; Sacco Perasso, C.; Ardizzone, G.; Gravina, M.F. Colonization dynamic on experimental limestone substrata: The role of encrusting epilithics favouring boring polychaetes. *Hydrobiologia* **2019**, *842*, 101–112. [[CrossRef](#)]
41. Ponti, M.; Fava, F.; Perlini, R.A.; Giovanardi, O.; Abbiati, M. Benthic assemblages on artificial reefs in the Northwestern Adriatic sea: Does structure type and age matter? *Mar. Environ. Res.* **2015**, *104*, 10–19. [[CrossRef](#)] [[PubMed](#)]
42. Antoniadou, C.; Voultziadou, E.; Chintiroglou, C. Benthic colonization and succession on temperate sublittoral rocky cliffs. *J. Exp. Mar. Biol. Ecol.* **2010**, *382*, 145–153. [[CrossRef](#)]
43. Choi, D.R. Ecological succession of reef cavity-dwellers (Coelobites) in Coral Rubble. *Bull. Mar. Sci.* **1984**, *31*, 72–79.
44. Gili, J.M.; Ballesteros, E. Structure of Cnidarian populations in Mediterranean sublittoral benthic communities as a result of adaptation to different environmental conditions. *Oecologia Aquat.* **1991**, *10*, 243–254.
45. Harms, J.; Anger, K. Seasonal, annual, and spatial variation in the development of hard bottom communities. *Helgoländer Meeresunters.* **1983**, *36*, 137–150. [[CrossRef](#)]
46. Fariñas-Franco, J.M.; Roberts, D. Early faunal successional patterns in artificial reefs used for restoration of impacted biogenic habitats. *Hydrobiologia* **2014**, *727*, 75–94. [[CrossRef](#)]
47. Toledo, M.-I.; Torres, P.; Díaz, C.; Zamora, V.; López, J.; Olivares, G. Ecological succession of benthic organisms on niche-type artificial reefs. *Ecol. Process.* **2020**, *9*, 38. [[CrossRef](#)]
48. Dean, T.A.; Hurd, L.E. Development in an estuarine fouling community: The influence of early colonists on later arrivals. *Oecologia* **1980**, *46*, 295–301. [[CrossRef](#)]
49. Herbert, R.J.H.; Collins, K.; Mallinson, J.; Hall, A.E.; Pegg, J.; Ross, K.; Clarke, L.; Clements, T. Epibenthic and mobile species colonisation of a geotextile artificial surf reef on the south coast of England. *PLoS ONE* **2017**, *12*, e0184100. [[CrossRef](#)] [[PubMed](#)]
50. Bastida, R.; Grosso, M.; Elkin, D. The role of benthic communities and environmental agents in the formation of underwater archaeological sites. In *Underwater and maritime archaeology in Latin America and the Caribbean*; Leshikar-Denton, M.E., Luna Erreguerena, P., Eds.; One world archaeology series; Left Coast Press: Walnut Creek, CA, USA, 2008; pp. 173–185. ISBN 978-1-59874-262-6.
51. Connell, J.H.; Slatyer, R.O. Mechanisms of succession in natural communities and their role in community stability and organization. *Am. Nat.* **1977**, *111*, 1119–1144. [[CrossRef](#)]
52. Coll, M.; Piroddi, C.; Albouy, C.; Ben Rais Lasram, F.; Cheung, W.W.L.; Christensen, V.; Karpouzi, V.S.; Guilhaumon, F.; Mouillot, D.; Paleczny, M.; et al. The Mediterranean sea under Siege: Spatial overlap between marine biodiversity, cumulative threats and marine reserves. *Glob. Ecol. Biogeogr.* **2012**, *21*, 465–480. [[CrossRef](#)]
53. Cahill, A.E.; Pearman, J.K.; Borja, A.; Carugati, L.; Carvalho, S.; Danovaro, R.; Dashfield, S.; David, R.; Féral, J.-P.; Olenin, S.; et al. A Comparative analysis of metabarcoding and morphology-based identification of benthic communities across different regional. *Seas Ecol. Evol.* **2018**, *8*, 8908–8920. [[CrossRef](#)] [[PubMed](#)]
54. Dobretsov, S.; Rittschof, D. Love at first taste: Induction of larval settlement by marine microbes. *Int. J. Mol. Sci.* **2020**, *21*, 731. [[CrossRef](#)]
55. Olabarria, C. Role of colonization in spatio-temporal patchiness of microgastropods in coralline turf habitat. *J. Exp. Mar. Biol. Ecol.* **2002**, *274*, 121–140. [[CrossRef](#)]
56. Underwood, A.J.; Chapman, M.G. Early development of subtidal macrofaunal assemblages: Relationships to period and timing of colonization. *J. Exp. Mar. Biol. Ecol.* **2006**, *330*, 221–233. [[CrossRef](#)]
57. Antoniadou, C.; Koutsoubas, D.; Chintiroglou, C.C. Mollusca Fauna from Infralittoral Hard Substrate Assemblages in the North Aegean Sea. *Belg. J. Zool.* **2005**, *135*, 119–126.
58. Poursanidis, D.; Koutsoubas, D.; Arvanitidis, C.; Chatzigeorgiou, G. ReefMedMol: Mollusca from the Infralittoral Rocky Shores—The biocoenosis of photophilic algae—In the Mediterranean Sea. *Biodivers. Data J.* **2016**, *4*, e7516. [[CrossRef](#)]
59. Poursanidis, D.; Chatzigeorgiou, G.; Dimitriadis, C.; Koutsoubas, D.; Arvanitidis, C. Testing the robustness of a coastal biodiversity data protocol in the Mediterranean: Insights from the molluscan assemblages from the sublittoral macroalgae communities. *Hydrobiologia* **2019**, *826*, 159–172. [[CrossRef](#)]
60. Russo, G.F.; Frascchetti, S.; Terlizzi, A. Population ecology and production of *Bittium latreillii* (Gastropoda, Cerithiidae) in a *Posidonia Oceanica* seagrass bed. *Ital. J. Zool.* **2002**, *69*, 215–222. [[CrossRef](#)]

61. Arvanitidis, C.; Bellan, G.; Drakopoulos, P.; Valavanis, V.; Dounas, C.; Koukouras, A.; Eleftheriou, A. Seascape biodiversity patterns along the mediterranean and the Black sea: Lessons from the biogeography of benthic polychaetes. *Mar. Ecol. Prog. Ser.* **2002**, *244*, 139–152. [[CrossRef](#)]
62. Giangrande, A.; Gravina, M.F. Brackish-water polychaetes, good descriptors of environmental changes in space and time. *Transit. Waters Bull.* **2015**, *9*, 42–55. [[CrossRef](#)]
63. Çinar, M.E.; Katagan, T.; Öztürk, B.; Bakir, K.; Dagli, E.; Açık, S.; Dogan, A.; Bitlis, B. Spatio-temporal distributions of zoobenthos in soft substratum of izmir bay (Aegean sea, Eastern Mediterranean), with special emphasis on alien species and ecological quality status. *J. Mar. Biol. Assoc. UK* **2012**, *92*, 1457–1477. [[CrossRef](#)]
64. Guerra-García, J.M.; Cabezas, M.P.; Baeza-Rojano, E.; García-Gómez, J.C. Spatial patterns and seasonal fluctuations of intertidal macroalgal assemblages from Tarifa Island, Southern Spain: Relationship with associated crustacea. *J. Mar. Biol. Assoc. UK* **2011**, *91*, 107–116. [[CrossRef](#)]
65. Keklikoglou, K.; Chatzigeorgiou, G.; Faulwetter, S.; Kalogeropoulou, V.; Plaiti, W.; Maidanou, M.; Dounas, C.; Lampadariou, N.; Arvanitidis, C. ‘Simple’ can be good, too: Testing three hard bottom sampling methods on macrobenthic and meiobenthic assemblages. *J. Mar. Biol. Assoc. UK* **2019**, *99*, 777–784. [[CrossRef](#)]
66. Azov, Y. Eastern Mediterranean—A Marine desert? *Mar. Pollut. Bull.* **1991**, *23*, 225–232. [[CrossRef](#)]
67. Voultziadou, E. Demosponge distribution in the Eastern Mediterranean: A NW–SE Gradient. *Helgol. Mar. Res.* **2005**, *59*, 237–251. [[CrossRef](#)]
68. Bianchi, C.N.; Azzola, A.; Parravicini, V.; Peirano, A.; Morri, C.; Montefalcone, M. Abrupt change in a subtidal rocky reef community coincided with a rapid acceleration of sea water warming. *Diversity* **2019**, *11*, 215. [[CrossRef](#)]