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# Structure and biodiversity of a Maltese maerl bed: New insight into the associated assemblage 24 years after the first investigation



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## ABSTRACT

Maerl beds are biogenic benthic habitats distributed worldwide and known to sustain high productivity and biodiversity levels. In the Mediterranean, the number of studies that have been carried out is limited, and little is known on its real distribution, mostly due to the difficulties of exploring such habitats — Due to the high transparency of Mediterranean waters, maerl can be found at depths of over 50 m making mandatory the use of benthic grabs and ROVs. The last published data from Maltese waters were taken two decades ago. In this present study, we provide new insights on this poorly known habitat, in particular regarding the north western bank, designated as a NATURA 2000 protected area, in which human activities are also carried out, such as blue fin tuna ranching. The objective of this work is to provide information regarding this delicate habitat, especially relevant for future management plans and authorities.

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## 1. Introduction

## A rhodolith bed is a sedimentary sea bed characterised by unattached non-geniculate calcareous red algae (Basso et al., 2016). The shape and structure of an individual rhodolith depends on environmental factors such as local hydrodynamics, bioturbation, depth, sediment grain size, nucleus density and biological factors, including algal species-related growth factors (Caragnano et al., 2016). Rhodolith beds generally have a very specific range of environmental tolerances due to their high sensitivity to burial and anoxia (Foster et al., 2013). Rhodolith beds have a global distribution and, in the Mediterranean Sea, these can be found on the submarine plateaux of the continental shelf around islands and capes (Foster, 2001). Despite occurring in shallow areas and lagoons within tropical seas, including the Red Sea (Pena and Barbara, 2010; Caragnano et al., 2016), in the

Mediterranean Sea, rhodolith beds are generally distributed at depths ranging between 30 m and 100 m (Aguiliar et al., 2009). The transparency of the water column above plays an important role in the magnitude of light irradiance which reaches the bed, and thus in the depth distribution of rhodolith beds in non-tropical seas, given that these beds normally occur in mesophotic zones (Cerrano et al., 2019). As a result, rhodolith beds show a patchy distribution across the Mediterranean Sea (Gambi et al., 2009).

A rhodolith bed can provide a wide spectrum of ecosystem services (Foster et al., 2013; Hall-Spencer et al., 2003), such as climate regulation through CO<sub>2</sub> uptake and primary production (Basso et al., 2016; Martin and Gattuso, 2009), and a contribution to the carbonate cycle of the continental shelf (Amado-Filho et al., 2012). The complex structure of rhodolith beds supports a rich biodiversity, enhancing the development of intricate food webs (Barbera et al., 2003; Sciberras et al., 2009; de Cerqueira Veras et al., 2020) and serving as a nursery for several commercially-important species (Foster et al., 2013). Rhodolith beds must be thought of as a non-renewable re-

Rhodolith beds must be thought of as a non-renewable resource (Barbera et al., 2003) due to their extremely low growth rate (1 mm per year) (Blake and Maggs, 2003). For this reason, the

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two main coralligenous species that characterise it, *Phymatolithon calcareum* (Pallas) W.H. Adey & D.L. McKibbin ex Woelkering & L.M. Irvine 1986 and *Lithothamnion corallioides* (P. Crouan and H. Crouan) P. Crouan and H. Crouan 1867, are included in Annex V of the EC Habitats Directive (92/43/EEC), and the corresponding habitat is listed under Annex V of the Marine Strategy Framework Directive (MSFD, -2008/56/EC).

Rhodolith bed integrity is threatened by human activities (Kamenos et al., 2003). The loss of habitat heterogeneity and habitat degradation may impact their functioning (Kamenos et al., 2003). In the past, rhodolith beds were exploited as a source of calcareous sediment to be used in agriculture (Birkett et al., 1998) and by bottom-fishing practices (Bordehore et al., 2003; Tauran et al., 2020). Aquaculture and off-shore farming can also significantly impact this benthic assemblage, increasing sedimentation rates and nutrients input (Hall-Spencer et al., 2006; Sanz-Lázaro et al., 2011). Since rhodolith beds are constituted by calcareous algae, they are also vulnerable to phenomena like ocean warming and acidification (Martin and Gattuso, 2009), with an array of physiological responses being recorded within rhodolith beds (Qui-Minet et al., 2019). The present knowledge gap concerning the real distribution of rhodolith beds in the Mediterranean currently constrains the implementation of a comprehensive conservation management plan for this habitat (Salomidi et al., 2012).

The Maltese archipelago is located within the Sicily Channel, which is a uniquely high-energy site at the crossroads between water masses originating in the Western and in the Eastern Mediterranean. The Modified Atlantic Mediterranean Water (MAW) stream flowing eastwards from the Straits of Gibraltar divides in two branches upon reaching the Tunisian coast, with the southern branch (the Atlantic-Ionian Stream), flowing between Sicily and Malta, creating a unique circulation system which enhances the establishment of coralligenous communities (Astraldi et al., 2001; Capodici et al., 2018; Innangi et al., 2019).

Two major rhodolith beds are known from Maltese offshore waters: with the most comprehensively explored being the north-east site covering an extensive marine area within the MT00000105 Natura 2000 (Special Area of Conservation of International Importance; (Borg et al., 1998), and a second bed located off the south-eastern coast of the island of Malta (Dimech et al., 2004). To date, there is a paucity of scientific literature on rhodolith bed investigations conducted within Maltese waters. The only comprehensive investigation of Maltese rhodolith beds, carried out in 1996 (BIOMAERL project), was published by Sciberras et al. (2009). More recently, in 2020, the Maltese Environment and Resources Authority (ERA) carried out the second national MSFD monitoring report within the same area (ERA, 2020), providing supplementary information regarding the human activities impacts which are likely affecting Maltese rhodoliths bed.

Here we presented novel insights on the north-eastern rhodolith bed located within Maltese coastal waters a still poorly known benthic assemblage, we also investigated factors putatively influencing rhodolith bed distribution patterns and the associated benthic community.

## 2. Materials and methods

In this current study, carried out within the framework of the HARMONY project (www.harmony-italiamalta.eu; funded through the INTERREG Italia-Malta 2014–2020 Programme), a considerable swathe of the Special Area of Conservation (SAC) of fil-Bahar bejn il-Ponta ta' San Dimitri (Ghawdex) u il-Qaliet (MT 00000105 Natura 2000; Fig. 1) was investigated. This area hosts the Maltese North Aquaculture Zone which involves intensive bluefin tuna (*Thynnus tunnus*) ranching activities which started operating after the BIOMAERL exploration (Sciberras et al.,

2009)but before this present study. The sites of this study are also located in the near proximity of a bunkering area which covers an extensive area of the northern Maltese waters.

Remotely Operated Vehicle (ROV; VVL-V400-4T model) dives were conducted between July and August 2020 from a vessel equipped with side-scan sonar (SSS). A van Veen benthic grab was deployed in order to collect macrobenthic samples, while megabenthic fauna was surveyed by the collected video footage from ROV transects.

The sampling sites were selected by replicating some of the stations previously surveyed by Sciberras et al. (2009); BIOMAERL project) and by also sampling from new stations surveyed by the Environment and Resources Authority (ERA, 2020) as part of the second national monitoring tranche linked with the MSFD (Marine Strategy Framework Directive, 2008/56/EC) obligations. The stations selected within this study were chosen based on the presence of high density rhodolith beds identified during the previous exploration conducted by ERA. When possible, three replicate samples were collected for each station, using a 0.1 m<sup>2</sup>, 0.005 m<sup>3</sup> van Veen grab (the coordinates of the sampling stations are listed in Appendix A, whilst their geographical location is shown in Fig. 1, which also includes the closest benthic sites explored by (Sciberras et al., 2009)).

Sediment sub-samples (100 g) were extracted from the internal part of the grab for sediment grain size analysis. The mean, modal and median (D50) particle size, as well as the interquartile deviation (D75–D25), sorting coefficient, kurtosis, skewness and the proportions of gravel, sand and mud were calculated for the sediment samples using GRADISTAT v.4 software (Blott and Pye, 2001).

The grab samples were sieved through a 500  $\mu$ m mesh and subjected to macrofaunal sorting processes. All the algal and macrofaunal specimens were identified to the lowest possible taxonomical level (when the species could not be determined, a higher taxonomical rank was assigned e.g. genus or family). A total of twenty grab samples collected from the eight sampling stations, taken at a depth ranging between 63 and 85 m (Appendix A). For stations 1 and 6, only one sample was successfully collected, due to the limitations of rough weather conditions and the local low-density presence of rhodoliths. Rhodolith and other macro-algal surfaces were also examined for any epibiotic species colonising their surfaces. For crustaceans, polychaetes and sipunculids, only individuals with a cephalic portion were recorded. For molluscs, only shells still containing soft tissues were recorded, while for bryozoans whole colonies were recorded as single units. Every species of the macro-algal community was also wet-weighed and identified; for the species Flabellia petiolata, only the laminae were weighed given that rhizoids embed a large amount of sediment. All the coralligenous hard structures were separated and, in order to compare the results obtained with those obtained from the previous study conducted in the same area, were classified within six morphotype classes (from A to F) as defined by Sciberras et al. (2009). Each morphological class was finally quantified in terms of dry weight, Given its influence on habitat complexity, the presence or absence of Flabellia petiolata was used a posteriori within statistical analyses in order to investigate any differences between assemblages. Six diversity indices were computed (Table 1): species richness (S); number of individuals (N); Simpson's index (D); Eveness (J'); Shannon's index (H') and Gini–Simpson's index  $(1-\lambda')$ . All the identified species were assigned to six different trophic groups, species whose trophic status was not represented in the literature were grouped within the "unknown" category. Multivariate analysis was carried out by using PRIMER v.7 (Clarke and Gorley, 2015) after a square root transformation of the raw matrix of individual abundances and resemblance values into a Bray-Curtis



Fig. 1. Map representing all the sites (grey circles) sampled during summer 2020 through a Van Veen grab. The coordinates of the same sampling sites are listed in Appendix A along with the respective depth at each site. Black triangles represent the closest sites sampled from Sciberras et al. (2009) during the BIOMAERL campaign, at which live calcareous algae were recorded. The green polygons represent the bunkering area on site; pink polygons represent off-shore aquaculture facilities.



**Fig. 2.** Frequency histogram with amount of living rhodoliths (gr) sampled per each station divided by morph-types with relative standard error bars.

similarity matrix. The PERMANOVA test (Anderson, 2014) was used to check for differences among the assemblages for the factor presence/absence of *F. petiolata* for all biodiversity indices, sediment sizes, maerl weights and compositions. The matrix of similarity was represented using a non-MultiDimensional Scaling (nMDS) technique.

## 3. Results

A total of 4546.13 g of living rhodolith was extracted from the grab samples. The overall mean mass of rhodolith per grab was 334.21 g  $\pm$  156.05, reaching a maximum mass of 765.02 g. The predominant rhodolith morpho-types encountered across all the samples belonged to morphs A (rhodolith with free-leaving branches; 35,9%) and morph D (rhodolith with very rough and rugged surface; 35.86%), followed by morph E (14.78%) and F (9.88%); morph C and morph B were generally rare or completely absent within some grab samples, with an average percentage composition value of 2.70% and 0.84%, respectively (Fig. 2).

The superficial sediment of the sampled seabed was mainly detrital, consisting of fragmented rhodoliths, empty shells, sea urchin spines, polychaete tube debris and foraminifera. The interstitial sediment was poorly sorted, dominated by gravelly sand (>500  $\mu m)$  represented by an average percentage composition of gravel (15.2%  $\pm$  2.32), sand (79.6%  $\pm$  2.77) and mud (5.2%  $\pm$  0.8; Appendix B).

A total of 197 species were collectively identified across all the samples and through the visual analysis of the collected ROV videos. In particular, 8 algal species, 188 invertebrate (6 belonging to megafauna) and one chordate species made up the full identified species complement (Appendix C for the list of all the species). Looking at the wet weight, the algal assemblage was dominated by Flabellia petiolata (58.0%), followed by Zonaria tournefortii (38.1%) (Table 2). Despite its high abundance, F. petiolata was only recorded in 12 of the 20 collected grab samples, contrary to Z. tournefortii which was recorded within all the grab samples. The dominant taxa in terms of species richness and individual abundance were crustaceans and polychaetes (69 and 66 species, respectively). Crustacea had the highest individual abundance (53.1%) and species richness (37.5%) values among all the samples (see Table 3). Polychaetes exhibited a similar species richness value as crustaceans (35.9%) but less than half of their individual abundance value (25.2%). Species belonging to Mollusca, Sipunculida, Bryozoa, Echinodermata, Chordata, Brachiopoda and Pantopoda were also recorded within the grab samples (Table 3). A list of species dominance representing at least 80% of the total individual abundance for the overall assemblage sampled during this study is provided in Table 5. The dominant species in terms of individual abundance were Chondrochelia savignyi (Tanaidacea), representing 4.51% of all the cumulative individual abundance, followed by Leptocheirus pectinatus (4.15%) (Amphipoda) and Apionsoma misakianum (4,06%) (Sipunculida). A total of sixty-nine recorded crustacean species belonged to seven different orders, represented mostly by Amphipoda (42.4% of the crustacean species) followed by Decapoda, Isopoda and Tanaidacea. In terms of individual abundances, besides amphipods (51.2% of the crustacean abundances), tanaids were the most represented taxon (16.1% of the crustacean abundances) (Table 4). With respect to molluscs, 22 different species were identified; Polyplacophora was the group with the highest individual abundance and species richness values (44.8% and 40.9% of mollusc species richness and abundance, respectively), followed by Bivalva and Gastropoda (Table 4).

Deposit feeders were by far the dominant trophic group in terms of species richness (42%) and individual abundance (58%),

#### Table 1

Traditional biodiversity indices for the eight stations object of this study with relative standard error when applicable Species richness: S; Number of individuals: N; Simpson's index: D; Eveness: J'; Shannon's index: H'; Gini–Simpson's index:  $1-\lambda'$ . Are also included mean sediment size and IQR values (in micron and phi in brackets) for the relative stations.

	S	N	D	J'	H'	1-Lamba'	Mean	IQR (D75-D25)
Station 1	14.00	23.00	4.15	0.95	2.51	0.95	684.03 (1.25)	823.93 (2.20)
Station 2	37.33 ± 4.91	$87 \pm 17$	$8.15 \pm 0.82$	$0.9\pm0.01$	$3.22\pm0.12$	$0.95\pm0.008$	760.91 (1.52)	1874.43 (3.83)
Station 3	$17.67 \pm 2.85$	$28.67 \pm 6.33$	$4.98\pm0.51$	$0.94\pm0.01$	$2.67\pm0.20$	$0.95\pm0.015$	845.16 (1.21)	1038.64 (2.62)
Station 4	$33.33 \pm 5.36$	$60.67 \pm 14.88$	$7.9\pm0.82$	$0.95\pm0.01$	$3.29\pm0.14$	$0.97\pm0.004$	732.66 (1.49)	939.00 (2.67)
Station 5	$39 \pm 5.57$	75.33 ± 15.86	$8.8\pm0.86$	$0.94 \pm 0.005$	$3.42\pm0.14$	$0.97\pm0.004$	768.67 (1.26)	787.52 (2.14)
Station 6	26.00	37.00	6.92	0.97	3.16	0.98	1169.77 (0.45)	1684.57 (2.35)
Station 7	$25.67 \pm 3.76$	$54.33 \pm 18.56$	$6.28\pm0.52$	$0.95\pm0.01$	$3.05 \pm 0.12$	$0.97 \pm 0.005$	909.34 (1.22)	1301.78 (3.11)
Station 8	$23 \pm 6.24$	$54\pm20.40$	$5.51 \pm 1.04$	$0.9\pm0.03$	$2.75 \pm 0.24$	$0.92\pm0.014$	867.88 (0.95)	1101.33 (-4.72)

#### Table 2

List of the algal species found across all the samples and their % contribute in terms of wet biomass.

Algal species	% Wet weight
Flabellia petiolata	58.0%
Zonaria tournefortii	38.1%
Peyssonelia sp.	1.9%
Valonia utricularis	1.3%
Dictyota dicotoma	0.3%
Cryptonemia sp.	0.2%
Gelidium sp.	0.1%
Halopteris filicina	0.1%

#### Table 3

List of the Phyla found across all the samples with relative contribute (%) in terms of abundances and species richness.

	% Abundance	% Species
Crustacea	53.1%	37.5%
Polychaeta	25.2%	35.9%
Mollusca	7.2%	12.0%
Sipunculida	6.3%	4.3%
Bryozoa	5.4%	6.0%
Echinodermata	2.3%	2.7%
Chordata	0.2%	0.5%
Brachiopoda	0.2%	0.5%
Pantopoda	0.1%	0.5%

### Table 4

List of the Orders of Mollusca found across all the samples with relative contribute (%) in terms of abundances and species richness. List of the Orders of Crustacea found across all the samples with relative contribute (%) in terms of abundances and species richness.

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Mollusca	% abundance	% species
Polyplacophora	44.8%	40.9%
Bivalva	28.7%	31.8%
Gastropoda	25.3%	22.7%
Scafopoda	1.1%	4.5%
Crustacea		
Amphipoda	51.2%	42.4%
Tanaidacea	16.1%	12.1%
Isopoda	15.8%	18.2%
Decapoda	12.0%	22.7%
Mysidacea	3.4%	1.5%
Ostracoda	1.1%	1.5%
Copepoda	0.5%	1.5%

followed by predators (20% of the species and 14% of the total abundance of individuals). On the other hand, grazers were the least represented trophic group, representing only 7% of the assemblage in terms of individual abundance (Table 6). The species *Chondrochelia savignyi, Leptocheirus pectinatus, Apionsoma misakianum, Joeropsis brevicornis,* and *Maera sodalis* (Amphipoda) were the most abundant deposit feeders and together contributed to 31% of the cumulative deposit feeder abundance. The most

common deposit feeders were amphipods and sipunculids. Polychaetes and crustaceans were the most abundant among the recorded predator species. For instance, *Eurydice truncata, Glycera convoluta* and *Paucibranchia fallax* contributed up to 32% of the corresponding cumulative individual abundance. Suspension feeders were mainly represented by bryozoans, with *Tubulipora sp., Beania magellanica* and *Reteporella aporosa* contributing to 38% of their total individual abundance. The 67% of the omnivore assemblage individual abundance was represented by *Syllis prolifera* (Polychaeta), *Munida tenuimana* (Decapoda) and *Syllis gracilis* (Polychaeta). Ultimately, the macro grazer fauna belonged mostly to the Polyplacophora group, followed by representatives from Echinodermata and Gastropoda. For instance, *Chiton corallinus, Bittium latreillii* and *Genocidaris maculata* contributed to 59% of the cumulative grazer individual abundance.

From the analysis of the ROV videos, six megafaunal species belonging to the Echinodermata phylum were identified. The most common species was Spatangus purpureus, recorded in almost all the ROV videos, followed by Centrostephanus longispinus and Stylocidaris affinis. A dense aggregation of Spaerechinus granularis was recorded at a number of surveyed stations. A single specimen of Antedon mediterranea was recorded. Two mega predators were also recorded and identified: Astropecten sp. and Luidia ciliaris. From the analysis of the collected ROV video footage, two main distribution patterns of the recorded rhodolith accumulations could be identified: i) a low-density rhodolith bed under the influence of bottom currents, with rhodoliths accumulated in the ditches between ripple marks (Micallef et al., 2012) (Fig. 3) and (ii) a high-density rhodolith bed with a patchy distribution defined by the bioturbation activity of crawling/burrowing megafaunal species which include Spatangus purpureus (Lohrer et al., 2004; Barberá et al., 2011; Demestre et al., 2017) (Fig. 3). The results of the PERMANOVA test showed significant differences between those grabs samples dominated by F. petiolata and those without (p < 0.05) (Fig. 4). No differences were found among the diversity indexes, rhodoliths biomass and composition and sediment size for the factor presence/absence of F. petiolata.

## 4. Discussion

The occurrence of oligotrophic, highly-transparent waters around the Maltese Islands is corroborated by the corresponding occurrence of accumulations of rhodoliths at a maximum depth of 85 m (Sciberras et al., 2009). No significant differences were found between the two identified rhodolith distribution patterns in terms of rhodolith morpho-type composition. Our analysis has shown that the two dominant rhodolith morpho-types were the A (rhodolith with free-leaving branches) and the D (rhodolith with very rough and rugged surface) ones within both distribution patterns. The high fragility of morpho-type A suggests that these rhodolith beds are not affected by strong currents, allowing the development of thin, branches-like structures (Marcack, 1999; Rendina et al., 2020a). On the other hand, this morpho-type was

#### Table 5

List of the species contributing to describe at least 80% of the macro faunal assemblage with relative percentage contribute in terms of abundances and species richness. For each species is described listed their belonging taxonomical group: A: Amphipoda; B: Bryozoa; Bi: Bivalva; C: Polyplacophora; D: Decapoda; E: Echinodermata; G: Gastropoda; I: Isopoda; O: Ostracoda; P: Polychaete; S: Sipunculida; T: Tanaidacea.

Species		% abundance	% abundance taxa	Species		% abundance	% abundance taxa
Chondrochelia savignyi	Т	4.51%	48.54%	Anapagurus sp.	D	0.81%	11.69%
Leptocheirus pectinatus	Α	4.15%	14.02%	Athanas nitescens	D	0.81%	11.69%
Apionsoma misakianum	S	4.06%	59.21%	Cymodoce truncata	Ι	0.81%	8.91%
Syllis prolifera	Р	3.34%	12.13%	Lumbrineris geldiayi	Р	0.81%	2.95%
Lysianassa costae	Α	2.61%	8.84%	Gregariella sp.	Bi	0.81%	36.00%
Maera sodalis	Α	2.61%	8.84%	Notomastus profundus	Р	0.72%	2.62%
Joeropsis brevicornis	I	2.61%	28.71%	Pista cristata	Р	0.72%	2.62%
Paranthura sp.	I	2.43%	26.73%	Schistomeringos rudolphy	Р	0.72%	2.62%
Neogammarus sp.	Α	2.43%	8.23%	Patinella radiata	В	0.72%	12.31%
Apseudes sp.	Т	2.34%	25.24%	Armandia cirrhosa	Р	0.63%	2.30%
Munida tenuimana	D	2.25%	32.47%	Leucothoe euryonix	Α	0.63%	2.13%
Syllis gracilis	Р	2.07%	7.54%	Lysianassa longicornis	Α	0.63%	2.13%
Mysidiacea sp.	Μ	1.98%	100.00%	Ostracoda sp.	0	0.63%	100.00%
Lysidice unicornis	Р	1.71%	6.23%	Callochiton laevis	С	0.63%	17.95%
Eurydice truncata	Ι	1.71%	18.81%	Stylocidaris affinis	Е	0.63%	25.00%
Apherus bispinosa?	Α	1.62%	5.49%	Lumbrinidae sp.	Р	0.63%	2.30%
Apseudes talpa	Т	1.62%	17.48%	Disconectes picardi	Ι	0.54%	5.94%
Chiton corallinus	С	1.62%	46.15%	Harpinia antennaria	Α	0.54%	1.83%
Maera grossimana	Α	1.44%	4.88%	Maera sp.	Α	0.54%	1.83%
Bittium latreillii	G	1.35%	68.18%	Processa sp.	А	0.54%	1.83%
Glycera convoluta	Р	1.26%	4.59%	Aspidosiphon muelleri muelleri	S	0.54%	7.89%
Paucibranchia fallax	Р	1.26%	4.59%	Nephasoma rimicola	S	0.54%	7.89%
Onchnesoma steenstrupii steenstrupii	S	1.17%	17.11%	Fenestrulina sp.	В	0.54%	9.23%
Alpheus macrocheles	D	1.17%	16.88%	Euclymene sp.	Р	0.45%	1.64%
Tubulipora sp.	В	1.17%	20.00%	Magelonidae sp.	Р	0.45%	1.64%
Psamathe fusca	Р	1.08%	3.93%	Amphitoe raimondi	А	0.45%	1.52%
Spiophanes kroyeri	Р	1.08%	3.93%	Syllis alternata	Р	0.45%	1.64%
Beania magellanica	В	1.08%	18.46%	Dorvillea sp.	Р	0.45%	1.64%
Reteporella aporosa	В	0.99%	16.92%	Goniada exadentes	Р	0.45%	1.64%
Limatula subauricolata	Bi	0.90%	40.00%	Amphipholis squamata	Е	0.45%	17.86%
Genocidaris maculata	Е	0.90%	35.71%	Ophiopsila aranea	Е	0.45%	17.86%
Lysidice ninetta	Р	0.90%	3.28%	Crisia sp.	В	0.45%	7.69%

Table 6

List of identified	trophic gr	oups and t	their co	ontribute o	n des	cribing the	overall	assemblage.	Raw	and
percentage quan	tities are g	given for s	pecies	abundance	es and	l richness.				

Trophic Group	Number of speci	es	Abundance	
	Raw	Percentage	Raw	Percentage
Deposit feeder	542	49%	63	34%
Predator	149	13%	35	19%
Suspension feeder	96	9%	26	14%
Omnivore	126	11%	20	10%
Scavenger	106	10%	15	8%
Grazer	73	7%	14	8%
Unknown	16	1%	11	6%

often found strongly embedded by F. petiolata rhizoids, which presumably represent a further protection against fragmentation. Morpho-type D was mainly composed of coarse sediment covered by coralligenous algae, forming nucleated rhodolithes showing signs of branching. The latter observation could potentially be explained in terms of a semi-burrowed position occupied by the deeper layer of this rhodolith bed. The observed rhodolith morpho-type distribution is not consistent with that reported by Sciberras et al. (2009), within which the most abundant morpho-types were the D, E (open branching forms) and F (closed branching forms) ones, suggesting a higher degree of benthic hydrodynamism within the sites sampled in 1996 by the same authors (Marrack, 1999; Gagnon et al., 2012). The putative shift in hydrodynamic regime experienced by the sampled rhodolith bed over the interval between the two studies is also supported by the analysis of the collected sediment cores, for instance, the percentage of muds ranging from 2.4% to 10.3% recorded in the present study was higher than that those recorded in Sciberras et al. (2009) (0.3%~1.0%).

The biotic assemblage associated with the surveyed rhodolith bed was characterised by a poor algal diversity, with a substantial dominance by F. petiolata and Z. tournefortii. This result is drastically in contrast with results from the study by Sciberras et al. (2009), within which a total of eighty-five different algal species were recorded. This difference might be explained by the higher number of sampling stations as well as by the multi-seasonal sampling design of the BIOMAERL project. On the other hand, an active benthic grazing megafaunal community was recorded in this present study, composed mostly of echinoderm species. The abundance of grazer species such as *Centrostephanus* longispinus, Stylocidaris affinis and Spaerechinus granularis, generally observed in the current study within aggregations, might have significantly influenced the algal diversity and abundance recorded during the summer season during which our sampling activities were carried out (Guillou and Lumingas, 1998; Guillou et al., 2002). Moreover, Luidia ciliaris might also play an important role as a benthic. top predator by controlling the abundances of other echinoderm species, most notably Spatangus purpureus (Brun, 1972).

In contrast with the macrofloral community, the recorded benthic macrofaunal assemblage was characterised by a high biodiversity. The absence of differences of faunal assemblages related with the maerl biomass, despite inter-sample differences, is consistent with the findings of Sciberras ecal. (2009) and other



Fig. 3. Top: low density rhodolith bed under sustained currents cumulated in the ripple marks ditch. Bottom: low current rhodolith bed characterised by high density and patchy distribution with bioturbation activity of *Spatangus purpures* defining the intricate distribution pattern.



**Fig. 4.** non metric Multi-Dimensional Scaling (nMDS) showing graphically the difference of faunal benthic assemblages for the factor presence (F)/absence (NF) of *F. petiolata* within the samples after a square root transformation of the raw data. Stress degree is also indicated in the figure.

studies which demonstrated how the associated biota appears to be more dependent on the grain-size properties of the sediment derived from the fragmentation of the rhodoliths (De Grave et al., 2000; Rowe, 1990) rather than on the biomass of the rhodoliths themselves. In the other hand, significant differences were found between the assemblages for the factor presence/absence of *F. petiolata*, indicating that this species influences the assemblage, changing the degree of complexity of the substratum by providing new surfaces (laminae) and by aggregating sediment. The differences among these assemblages cannot be explained by diversity indices, rhodoliths biomass and composition and sediment size for which no significant differences were found.

The sampled rhodolith bed can be considered to support a number of different communities, namely: (i) interstitial and burrowing forms, including amphipods, polychaetes and sipunculidae; (ii) free-living crawling forms, including decapods and gastropods; and (iii) attached forms, including tubular polychaetes and bryozoans.

In our study, as well as in a number of others (e.g. Hinojosa-Arango and Riosmena-Rodríguez, 2004), the dominant recorded taxonomical groups were crustaceans, polychaetes and molluscs, although polychaetes are reported as the dominant taxon within other studies (e.g. Riera et al., 2012). The macrofaunal assemblage recorded within our study was extensively compared with that reported by Sciberras et al. (2009) and many substantial differences between the two are evident. Within the current study, the dominant group (in terms of individual abundance) among crustaceans were Amphipoda, followed by Tanaidacea and Isopoda, while Decapoda was second in terms of species richness. Chondrochelia savignyi was the overall dominant species in terms of individual abundance, followed by Leptocheirus pectinatus and Apionsoma misakianum. Surprisingly, a considerably higher number of species belonging to Sipunculida and Polyplacophora were recorded in this current study when compared with the Sciberras et al. (2009) study. Seventy-six new species for Maltese rhodolith beds were recorded and fully identified (to species level) in this present study (Appendix B), highlighting the significant knowledge gaps concerning this benthic assemblage, in particular for the Tanaidacea, Polyplacophora, Isopoda and Sipunculida taxa. On the other hand, anomalously low numbers of species and individuals for gastropods (except for Bittium latreillii) and bivalves were recorded in our study, partly consistent with the findings

of Castriota et al. (2005), who similarly report low degrees of gastropod diversity but not for bivalve diversity. This might be explained through a putative surge in sedimentation rates triggered by a less vigorous benthic hydrodynamic regime (Carvalho et al., 2020). Among molluscs, the dominant group in terms of individual abundance was the Polyplacophora one, of which five additional species have been recorded in this present study since the previous work (Table 4).

The trophic group analysis conducted in this current study also supports the sedimentation rate/hydrodynamic regime shift hypothesis. In fact, the dominant group, both in terms of species richness and individual abundance, was that composed by deposit feeders. This high diversity might represent a response of the benthic community to a high and heterogeneous Particulate Organic Matter (POM) input to the rhodolith bed being surveyed, which in turn might be caused by the proliferation of aquaculture facilities in recent years around the same rhodolith bed. A relatively low diversity and abundance of predators was recorded in this current study, especially considering the high availability of prey. This is not consistent with the findings of Sciberras et al. (2009), in which predators were the dominant group, followed by deposit feeders. The importance of macro grazers, represented mainly by Polyplacophora, within the surveyed rhodolith bed community, might be underestimated as a result of the low recorded values of individual abundance (Castriota et al., 2005). One could speculate that, in the surveyed rhodolith bed, due to recorded low diversity and biomass values for macroalgal species, chitons might be feeding mostly on microalgae (not assessed in the current study) growing on the surface of rhodoliths and on macroalgae (e.g. F. petiolata), thus controlling epiphytic species (Carvalho et al., 2020; Castriota et al., 2005; Legrand et al., 2020). Moreover, despite the belief that most of the identified amphipod species act as deposit feeders, the ability of some of these species to shift to grazing behaviour in relationship to food availability has been documented (Guerra-García et al., 2014). A similar trophic regime shift has been documented for some polychaetes, including Lysidice ninetta (Castriota et al., 2003). Bryozoa represented most of the suspension feeders growing on the surface of laminae of Flabellia petiolata and, sporadically, also on rhodolith surfaces.

Many authors have described maerl and rhodolith bed assemblages from different regions, highlighting substantial differences between them, thus suggesting a broad array of factors influencing the composition of the same assemblages (Amado-Filho et al., 2007; Riera et al., 2012; Carvalho et al., 2020; Navarro-Mayoral et al., 2020). The trophic regime shift observed for the surveyed rhodolith bed for the 1998–2020 period, putatively due to an increment of the benthic sedimentation rate, might have been triggered in turn by natural causes (e.g. hydrodynamic regime shift) or, alternatively, by anthropogenic drivers (Mangion et al., 2017b). The Bahar bejn Il-Ponta ta' San Dimitri (Ghawdex) u Il-Qaliet Natura 2000 hosts intensive aquaculture and bunkering activities, and, within this context, we suspect a recent alteration of this fragile circalittoral assemblage was mediated by changes in POM sedimentation rates (Hall-Spencer et al., 2006; Aguado-Giménez and Ruiz-Fernández, 2012) and possibly through changes in the chemical composition of the same sediment (Sanz-Lázaro et al., 2011; Mangion et al., 2014, 2017a,b) which in turn might have arisen as a result of an intensification of the previously mentioned activities in this area. These putative impacts were hinted at within Malta's second national MSFD monitoring report (ERA, 2020), which estimates that only 9.19% of the rhodolith bed in question is currently impinged upon by the same activities. Moreover, numerous lost fishing gears (mostly long lines) were recorded through our ROV explorative dives, suggesting an intensive pressure from fishing activities on benthic assemblages within the area (Hall-Spencer et al., 2003; Bordehore et al., 2003; Ferrigno et al., 2018; Rendina et al., 2020b; Mangano et al., 2017).

## 5. Conclusion

The findings of this current study suggest a putative change since 1998 in POM sedimentation rates for the upper circalittoral area supporting the surveyed rhodolith bed, possibly due to the installation of offshore bluefin tuna fattening cages in close proximity with the same bed. Only few targeted studies on the potential impact of these aquaculture facilities on the adjacent rhodolith beds have been conducted to date (Hall-Spencer et al., 2006; Sanz-Lázaro et al., 2011) and hence results from this present study can represent a useful baseline (for instance, in terms of Descriptor 1 of the EU's MSFD) as well as support calls for more in-depth impact assessment studies to be conducted in future. A more comprehensive multi-seasonal trophic, biogeochemical (e.g. composition and volumes of POM) and hydrodynamic assessment of the rhodolith bed under investigation needs to be conducted in the future in order to address the current extensive knowledge gap (e.g. the influence of bioturbation by megafaunal species on rhodolith distribution patterns) concerning such a productive benthic assemblage. We also advise a revision of the commonly-used sampling techniques for the macro benthic community. Indeed, despite the fact that the traditional van Veen grab allows for a quantitative investigation of sediment grain-size and infaunal parameters, it is subject to a number of practical limits. A specifically-designed dredge, although only generating a semi-quantitative dataset, can represent a solution for the sampling of sparsely-distributed rhodolith beds involving both macro and megafaunal species (Rees, 2009). In conclusion, more detailed studies are recommended in order to better understand the functioning of this fragile circalittoral ecosystem and in order to identify all the possible anthropogenic pressures and drivers on the same ecosystem pursuant to informing judicious environmental management measures.

## **CRediT authorship contribution statement**

**A. Deidun:** Funding acquisition, Conceptualization, Investigation, Methodology, Writing – original draft, Supervision. **A. Marrone:** Sample analysis, Software, Data curation, Writing – original draft. **A. Gauci:** Investigation, Methodology, Visualization. **J. Galdies:** Resources, Writing – review & editing. **M. Lorenti:** Resources, Validation. **M.C. Mangano:** Writing – review & editing, Funding acquisition, Supervision. **Karl Cutajar:** Investigation, Methodology, Visualization. **S. Mirto:** Funding acquisition, Supervision. **G. Sarà:** Writing – review & editing, Funding acquisition, Supervision.

## **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.rsma.2022.102262.

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