Spatial and bathymetric distribution of deepwater megabenthic echinoderms in the Malta 25-nautical miles Fisheries Management Zone

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Abstract

"Spatial and bathymetric distribution of deep-water megabenthic echinoderms in the Malta 25-nautical miles Fisheries Management Zone"

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Deep-sea benthic habitats around the Maltese islands are some of the least studied areas in the Mediterranean. Because of the limited research effort, inventories report a lower biodiversity in this area than in the surrounding regions, the Western Mediterranean, Aegean and Adriatic Sea. This is also the case for echinoderms; this marine phylum is generally well studied, but there is still a lack of data concerning deep-sea species. With the recent advent of remotely operated underwater vehicles (ROVs), the deep sea has become more accessible to scientists, especially rocky areas such as canyons, escarpments and cold-water coral reefs, which could not have been adequately sampled through trawling surveys. The aim of this study was to assess echinoderm diversity in deep Maltese waters, within the 25-nautical miles Fisheries Management Zone, through analysis of ROV video footage. In addition, the spatial and bathymetric distribution of echinoderms were described, as well as their density, preferred habitat and bottom types.

Video data were acquired through ROV surveys of Maltese benthic habitats as part of the Life Baħar for Natura 2000 project in the summers of 2015 and 2016. The bathymetric range covered by the videos used in the present study was from 216m to 1030m depth.

In total, 25 echinoderm taxa were identified, comprised of: 11 asteroids, 2 crinoids, 4 ophiuroids, 5 echinoids and 3 holothurians. Of these species, three were new records for Maltese waters: the sea stars *Marginaster capreensis*, *Sclerasterias neglecta*, and the holothurian *Mesothuria intestinalis*. Six species were observed deeper than their current accepted depth range in the Mediterranean. The most abundant species were the crinoids *Antedon mediterranea* and *Leptometra phalangium*, followed by the cidarids *Stylocidaris affinis* and *Cidaris cidaris*. Crinoids formed very dense aggregations of up to 2900 individuals/1000m^2, in a small area to the south of Malta. This area also hosts the only known Mediterranean population to date of the Atlantic sea star *Coronaster briareus*. Other echinoderms were more widely distributed across the studied area. Bathymetric distribution varied for each species, and the overall echinoderm diversity seemed stable across the surveyed depths. The preferred habitats also depended on each species, but rocky habitats dominated by deep-water corals were particularly diverse. Discarded anthropogenic objects proved to increase the diversity of sedimentary bottoms by providing colonizable hard substrata for sea stars, crinoids and sea urchins.

It can be concluded that the deep sea around the Maltese islands is a heterogeneous system hosting a diverse echinoderm fauna; however, there is still a lack of knowledge concerning the taxonomy and ecology of certain deep-sea echinoderms. The findings of the present study can be used to identify ecologically important areas for conservation purposes.

Résumé

"La distribution spatiale et bathymétrique des échinodermes mégabenthiques d'eau profonde dans la zone de gestion des pêches de 25 miles nautiques de Malte"

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Les habitats benthiques d'eau profonde autour des îles maltaises font partie des régions les moins étudiées en Méditerranée. À cause d'un effort de recherche limité, les recensements attribuent à la Méditerranée centrale une biodiversité moindre qu'aux régions alentours ; le bassin ouest, les mers Egée et Adriatique. C'est également le cas pour les échinodermes ; cet embranchement d'animaux marins est en général bien étudié, mais il y a encore un manque de données concernant les espèces des grands fonds. Avec l'arrivée récente des ROVs, petits robots sous-marins téléguidés, les grands fonds deviennent de plus en plus accessibles, en particulier les systèmes rocheux tels les canyons, escarpements et récifs coralliens d'eau froide, qui ne pouvaient pas être échantillonnés correctement par chalutage. L'objectif de cet étude était d'estimer la diversité en échinodermes dans les eaux maltaises de grande profondeur à travers l'analyse de vidéos prises par ROV. En plus, les distributions spatiale et bathymétriques, densités et habitats préférés des échinodermes furent décrits.

Les données vidéo furent collectionnées par le projet Life Baħar for Natura 2000 pendant les étés de 2015 et 2016, dans le cadre de leur étude des habitats benthiques maltais, délimitée par la zone de gestion des pêches de 25 miles nautiques de Malte.

Au total, 25 taxons d'échinodermes ont été identifiés, et comprenaient onze étoiles de mer, deux crinoïdes, quatre ophiures, cinq oursins et trois holothuries. Parmi ces espèces, trois furent trouvées pour la première fois en eau maltaise : les étoiles de mer *Marginaster capreensis*, *Sclerasterias neglecta*, et l'holothurie *Mesothuria intestinalis*. Six espèces furent observées à plus grandes profondeurs que leur limite actuelle en Méditerranée. Les espèces les plus abondantes étaient les crinoïdes *Antedon mediterranea* et *Leptometra phalangium*, suivis des oursins *Stylocidaris affinis* et *Cidaris cidaris*. Les crinoïdes formaient de très denses agrégations, dans une petite zone au sud de Malte. Cette zone abrite également la seule population méditerranéenne connue à ce jour de l'étoile de mer atlantique *Coronaster briareus.* Les autres espèces étaient plus largement distribués dans la région étudiée. La distribution bathymétrique variait selon l'espèce concernée, et la diversité globale en échinodermes semblait être constante parmi les gammes de profondeur échantillonnées. Les habitats privilégiés dépendaient également de l'espèce, mais les systèmes rocheux dominés par des coraux d'eau profonde étaient particulièrement diversifiés. Les objets d'origine anthropiques augmentaient la diversité des fonds sédimentaires en fournissant à diverses espèces des substrats durs à coloniser.

En conclusion, les grands fonds autour de l'archipel maltais s'avèrent être un système hétérogène abritant une faune diverse d'échinodermes. Par contre, il y a toujours un manque d'informations concernant la taxonomie et l'écologie de certaines espèces d'échinodermes d'eau profonde.

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FMZ: Fisheries Management Zone

ROV: Remotely Operated underwater Vehicle

HD: high definition

FAD: fish aggregating device

I. Introduction

1. Echinoderms

1.1 General Information

The echinoderms are a phylum of marine invertebrates consisting of roughly 7000 living species and divided into five extant classes: Asteroidea, Ophiuroidea, Echinoidea, Holothuroidea, and Crinoidea [1]. They are respectively more commonly known as sea stars, brittle stars, sea urchins, sea cucumbers, sea lilies and feather stars. They inhabit every ocean, in all climate zones and across all depth ranges [2]. Their characteristic features include a five-part radial symmetry, although this is not obvious in every class, as well as a highly developed water-vascular system and a calcite skeleton [1]. This skeleton appears in the dermis of the larva as individual calcareous plates, which eventually grow and fuse together to form a hard shell [3]. Holothuroids are an exception because their skeletal plates, called sclerites, do not merge and remain small-sized [3]. Their nervous system is non-centralized, which allows the equal perception of the environment from all sides [4].

With some exceptions, echinoderms all have separate sexes and fertilization is external [5]. Most species start off as bilaterally symmetric planktonic larvae, then undergo complex metamorphosis and become benthic adults, although a few holothuroids remain pelagic [6]. Other than some sedentary crinoids, all echinoderms are mobile, generally moving on the seabed or in the sediment [7]. Because of their lack of an osmoregulatory system, they are very sensitive to salinity changes; they are never present in freshwater and are rare in low-salinity areas such as the Baltic Sea [3][8]. This phylum displays various feeding habits, ranging from predators to scavengers, suspension feeders, deposit feeders, herbivores and detritivores [5].

Echinoderms play important roles in the ecology of marine environments [9], and many of them are keystone species [2]. Sea urchins are known to regulate algal populations, and predatory sea stars also strongly influence the structure of their ecosystem [2]. Noteworthy examples are the sea urchin *Diadema antillarum* in Caribbean coral reefs and the crown-of-thorn sea star *Acanthaster planci* in the Australian Great Barrier Reef [8]. Anthropogenic disturbances and diseases can cause large population density variations in echinoderms known as ‘outbreaks’ and ‘die-offs’, which have profound and cascading consequences on marine environments [2]. Crinoid beds, like those of *Leptometra phalangium*, can be indicators of highly productive areas and essential habitats for major commercial fish species [10]. Holothuroids, which are the dominant group in some deep sea ecosystems, play an important role in bioturbation and redistribution of detrital carbon [8]. In arctic ecosystems, the abundant ophiuroids are responsible for a large portion of the remineralization [8]. Because they are well-known and often conspicuous organisms, echinoderms are useful bio-indicators of the status of marine ecosystems [4].

In addition to their ecological importance, echinoderms also have a certain economic value. In the Mediterranean, the sea urchins *Paracentrotus lividus* and *Sphaerechinus granularis* are regularly fished and consumed [11]. Sea cucumbers of the class Holothuroidea are a highly valued delicacy in Asia, and overfishing has led to important declines in populations [12]. Some holothurians are frequently used as fishing bait, and sea stars are often sold as decorative items [11]. Because they are easily maintained in captivity and produce large quantities of eggs, echinoderms and especially sea urchins are excellent model organisms for embryological studies and molecular biology research [5]. Lastly, some holothurian species can have medical value because they produce compounds which have antimicrobial or anticoagulant properties [4].

1.2 Echinoderms in the Mediterranean

Located between Europe, Africa and Asia, the Mediterranean Sea constitutes the largest and deepest enclosed basin in the world [11]. It is connected to the Atlantic Ocean through the Strait of Gibraltar, to the Black Sea and the Sea of Marmara through the Bosphorus, and to the Red Sea through the Suez Canal [11]. The Strait of Sicily divides the Mediterranean into a western and an eastern basin [11]. The Mediterranean Sea is known to be a hotspot for biodiversity, with a high percentage of endemicity [13]. However, estimates of this diversity are still incomplete [11]. The Mediterranean benthos is considered to be relatively impoverished compared to that of the Atlantic Ocean [9].

Echinoderms in the Mediterranean represent 2.2% of the global echinoderm diversity; as of 2010, there were 154 known echinoderm species in the Mediterranean, including five introduced and three threatened species, but there was still a lack of data from southern and deeper areas [11]. Of these 154 echinoderm species, 24% are believed to be endemic to the Mediterranean [11]. This high percentage could be explained by their slow rate of dispersal and their brief pelagic larval stage [14]. After crustaceans and molluscs, echinoderms are the most diverse megabenthic invertebrates in the Mediterranean [13]. They are however much less diverse than in the Atlantic [13]. The variables that are most strongly correlated to echinoderm assemblages are salinity, temperature, primary productivity and pollution [8].

In general, due to a gradient of production, there is a decrease in biodiversity from the Northwest to the Southeast of the Mediterranean Sea [11]. For instance, in the Western Mediterranean, 144 echinoderm species are known, whereas the Levantine Sea hosts 73 echinoderm species [14]. Curiously, echinoderm inventories report a lower diversity in the Central Mediterranean than in the Adriatic Sea and the Aegean Sea, even though these areas are further away from the Western Mediterranean [14][15]. This is probably due to limited biodiversity studies in the Central Mediterranean [14][15]. In fact, species richness in an area is strongly correlated with the research effort in that area, and we should therefore be careful before comparing biodiversity in different parts of the Mediterranean Sea [13].

1.3. The Mediterranean deep sea

The present study will focus on the deep sea, that is, water below 200m depth, an area which is known to shelter some unique species and ecosystems [11][13]. The deep sea represents 65% of our planet's surface and 95% of the total biosphere, and is therefore the world's largest biome [13]. Compared to the first documented description of marine species that began in Ancient Greece with Aristotle, deep sea exploration is very recent, beginning at the end of the nineteenth century [11].

Due to narrow continental shelves, a large part of the Mediterranean classifies as deep sea, and it is characterized by a high salinity and a high homothermy with no thermal boundaries [11]. Danovaro et al. state that because trawling below 1000m is illegal in the Mediterranean, the deep benthic Mediterranean is the largest protected area worldwide [13]. The high heterogeneity of the deep seafloor and the associated abiotic conditions allow the establishment of a diverse fauna [16]. Deep sea biodiversity is similarly high in the eastern and western basin [12]. Yet the deep Mediterranean Sea remains largely unexplored, because of the difficulty in sampling [16]. Even if the Mediterranean is the one of the most studied regions, the deep sea has been more frequently studied in the Atlantic, Pacific and Arabian Sea [12]. According to Coll et al., compared to other ecosystems, the spatial distribution of benthos in the Mediterranean deep sea is very poorly known and in many cases only approximations are available [11]. Danovaro et al.'s extrapolations estimate that 66% of Mediterranean deep sea species are still unknown, not counting prokaryotes [13].

A common paradox is that even though food resources at greater depths are limited, the deep sea is highly diversified [17]. Some authors even suspect deep-sea biodiversity to be comparable to tropical rain forest biodiversity [17]. However, it is very delicate to try to compare 'shallow' and 'deep' water diversity as a whole, because it largely depends on the ocean and the taxon studied [17]. For instance, Mediterranean trends are different than those in the Atlantic, and while gastropods and cumaceans can show an increasing diversity with increasing depth, polychaetes and megafauna did not have a consistent link between depth and diversity in the Northwest Atlantic [17]. There are many theories that could explain an increasing diversity in the deep sea. The first one is that the stability in environmental conditions such as temperature, salinity, and oxygen allows the evolution of a diversified fauna while reducing potential disturbances [17]. This would favour species to occupy specialized niches, thus decreasing competition [17]. Another possible factor is the high heterogeneity of the deep seafloor and associated habitats [16]. Because of the limited food sources, deep-sea generalist species exploiting various food sources have an advantage over specialists [17]. As there is no real productivity *in situ* on deep-sea sediments, consumers depend on sinking organic matter from shallower waters [17]. Therefore, the abundance of deep sea benthos is strongly related to the quantity of food particles reaching these depths [17]. Because of the lower temperature in the deep sea, metabolic rates and turnover times are slower in deep-sea organisms, and they have longer lifespans [17]. Deep-sea organisms are also characterized by a low larval supply and high post-settlement mortality, which can slow down recovery rates after a disturbance [17]. All in all, diversity in the deep sea can be influenced by substratum, production, food sources, oxygen levels, currents and disturbances, which vary according to the region and can all have different impacts on each faunal group [13].

In the Mediterranean, the diversity is generally negatively correlated to increasing depth [17]. Mediterranean benthic biodiversity tends to be higher close to the coast and on continental shelves, and then decreases with depth, but there are some exceptions to this pattern [11]. Fauna associated with deep basins proves to be the least diverse, compared to that of canyons, open slopes and deep-water coral reefs [13]. Some authors consider the deep Mediterranean to be a 'biological desert', although diverse species are known even from the most oligotrophic deep areas of the Mediterranean [13]. It was previously believed that the level of endemism in the deep Mediterranean was much lower than in the Atlantic deep sea because of the Messinian crisis, the Gibraltar barrier and the higher deep sea temperatures, which are on average 10°C warmer in the Mediterranean than in the Atlantic at the same depth; however many studies tend to provide evidence contrary to this [11]. For instance, we now know that the Strait of Gibraltar is not impervious to deep sea macrobenthos, and that deep sea meiofauna is highly diversified [11]. Between 200m and 1000m depth, 13 to 15% of the biota are endemic macrobenthic species [11]. With the continuous discovery of new species, the affirmation that biodiversity in the Mediterranean deep sea is much lower than at the coast might prove incorrect [11].

Studies show that in the Mediterranean, the total biodiversity and abundance of echinoderms also appears to be inversely correlated to water depth [18]. In the deep Mediterranean, echinoderms are normally not dominant in terms of biomass, and poorly diversified [9]. This can be linked to temperature, light intensity, food availability and other factors influenced by depth [18]. The first Mediterranean deep sea explorations focused on dominant and commercial groups such as fishes and crustaceans, which are now well studied compared to other megafauna [16]. There have been relatively few studies on deep-sea echinoderms in the Mediterranean, and morphological descriptions are very limited [16]. Until recently, most information concerning deep-sea echinoderm species came from incidental catches in trawl surveys or oceanographic cruises [18]. The endemicity of deep sea Mediterranean echinoderms is still debated; they might be a sub-population of Atlantic species, unless the shallow Strait of Gibraltar and the higher temperatures of the deep Mediterranean act as a barrier for the immigration of echinoderm larvae from the Atlantic [16].

1.4. Echinoderm fauna of the Maltese islands

According to Terribile et al., some of the least studied regions of the Mediterranean are the deep-sea benthic habitats around the archipelago of Malta, in the Central Mediterranean [18]. Thus, knowledge on these deep sea assemblages is still incomplete, while there is a large amount of information available for coastal ecosystems [18]. Terribile et al. found that both diversity and abundance of benthic species were negatively correlated with depth around the Maltese islands [18].

As of 2006, 70 different species of echinoderms were known from Maltese waters [19]. Since then, this list has been updated by seven species in various published works. In 2007, the MARCOS research cruise of the RV *Urania* surveyed deep-sea ecosystems in the South Central Mediterranean Sea, in particular the recently discovered *Lophelia* coral reefs to the South of Malta, in order to assess their biodiversity [20]. From the bottom samples of this cruise, Mastrototaro and Mifsud recorded the sea star *Sclerasterias richardi* for the first time in Maltese waters [21]. Three species were added later on to the Maltese checklist from the same source; *Odontaster mediterraneus, Luidia sarsii* and *Ophiotreta valenciennesi rufescens*; the latter species was recorded for the first time in the Mediterranean through this study [20]. The Mediterranean International Trawl Survey (MEDITS), taking place in every EU Mediterranean Member State, is a programme designed to gather information on distribution, relative abundance and demographic structure of commercial demersal species [18]. However, benthic species are also caught in the process, and through the MEDITS data of 2009 and 2010, Terribile et al. were able to add *Hymenodiscus coronata* and *Ophiothrix quinquemaculata* to the checklist of Maltese echinoderms [18]. In 2016, Evans et al. published a paper on the first occurrence of the Atlantic sea star *Coronaster briareus* in the Mediterranean, based on video data gathered by ROV in 2015 and 2016 through the Life Baħar for Natura 2000 programme [15]. This is the same video data which this study will focus on. These updates result in a total of 77 echinoderm species for Malta, which represents 50% of all Mediterranean echinoderms.

Considering the central position of Malta in the Mediterranean, one would not expect it to have a considerably lower echinoderm diversity than the surrounding areas [22]. Thus, a greater research effort would probably lead to a better assessment of the total echinoderm diversity in Maltese waters. With their key position, the Maltese islands can be an ideal location to monitor the evolution of biodiversity patterns in the Mediterranean [18].

2. ROV exploration

In the past, most deep-sea biodiversity investigations have occurred in areas with loose sedimentary bottoms, because these are easily sampled with trawls and dredges [15]. Even nowadays, trawling surveys still allow new discoveries of Mediterranean benthos. An example is the Atlantic sea urchin *Gracilechinus elegans*, which was recorded for the first time in the Mediterranean by Mecho et al. in 2014, along with two very rare species of holothurians [16]. However, deep-sea rocky areas were not accessible for adequate sampling until the arrival of remotely operated underwater vehicles (ROVs) [15]. This method now enables biologists to easily survey the deep ocean without disturbing wildlife, and to access underwater caves, steep escarpments and canyons. With these technological advancements, the deep-sea benthos of the Mediterranean has been the focus of an increasing number of studies [11]. In addition to diversity assessments, ROV surveys can also be used to study the behaviour, habitat and assemblages of deep-sea species [15].

Recently, ROV exploration in the Tyrrhenian Sea led to the discovery of a large-scale crinoid facies with an absolute dominance of *Leptometra phalangium* in dense aggregations on coarse detrital bottoms, between 108m and 132m depth [23]. Other such non-destructive studies have revealed a great richness of corals and sponges in the deep Mediterranean Sea, which enrich the biodiversity of their ecosystem by providing colonizable substrata and refuges for other organisms [24]. While recent ROV studies tend to focus on deep-water coral reefs, very few have led to new information on echinoderm diversity [15]. Undoubtedly, be it through ROV exploration or not, the assessment of biodiversity is useful to understand ecosystem patterns and especially important for management and conservation purposes [8]. Without basic knowledge on species biology and diversity, it is impossible to successfully plan for the conservation of an area [18]. With the help of improved technologies, the deep sea becomes more accessible to comprehensive studies, and thus our knowledge on deep sea biodiversity has grown considerably in the last 20 years [11].

3. Concerning the present study

3.1. Area of study: The Malta 25 nautical miles Fisheries Management Zone

The area of the present study is the Malta 25 nautical miles Fisheries Management Zone (FMZ), represented in Fig. 1 by a large oval, with a total area of 6735 km^2 [25]. It was first established by Malta in 1971 in accordance with the United Nations Convention of the Law of the Sea, as an "Exclusive Fishing Zone" [25]. In 2004, it became the present Fisheries Management Zone through the European Union's Council Regulation (EC) No 813/2004, and only authorises vessels smaller than 12m to fish in this area [26]. These vessels are considered the more sustainable fishing segment and having minimal impact on marine environments [26]. The FMZ serves to protect the ever declining fish resources around the Maltese islands [26].

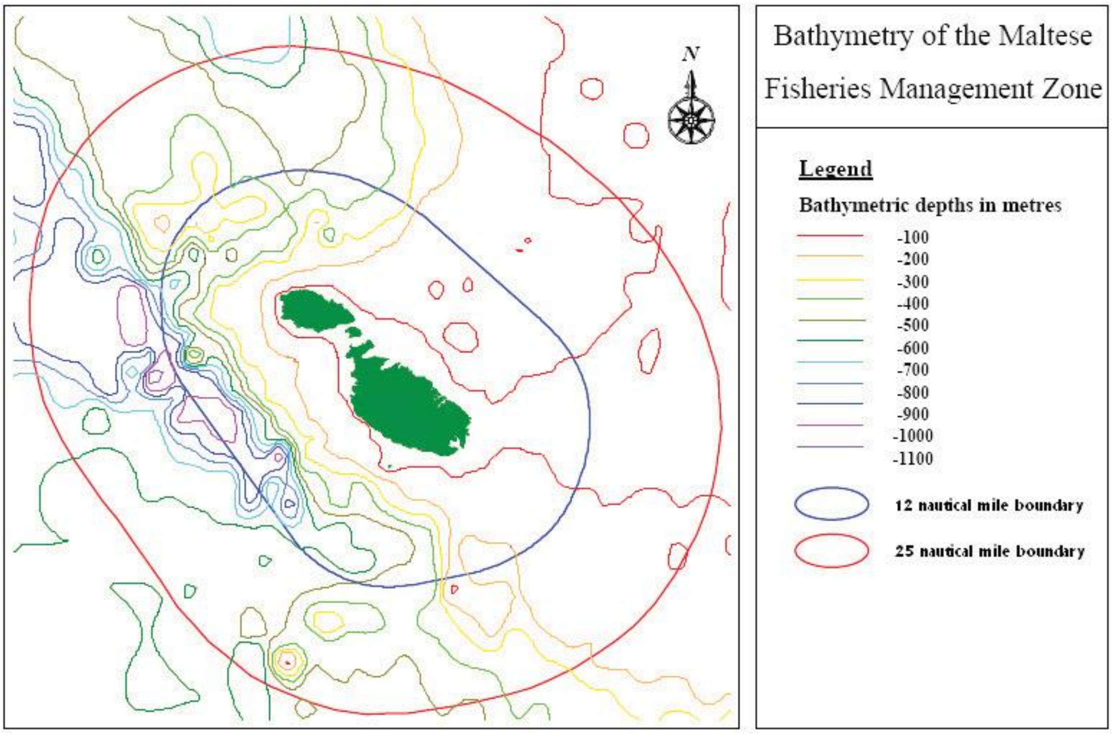


Fig. 1 : Map of Malta showing the 25 nautical miles Fisheries Management Zone (large oval) and the bathymetry [27]

The Maltese islands lie on the southernmost tip of the Sicilian continental shelf and display a highly irregular seabed topography [27]. The deepest trench in the Sicily Channel is the Malta Graben, to the west of the Maltese islands, reaching depths of 1650m [27]. To the northeast of Malta, Hurds Bank forms a shallow plateau of around 50m depth (Fig. 2) [27]. A study by Muscat has identified this shallow zone as a hotspot for echinoderms [22]. The proximity to the coast could induce abundant organic matter brought through runoff from the land, and the coralligenous and rhodolith habitats in this area are known to host a large biodiversity [22].

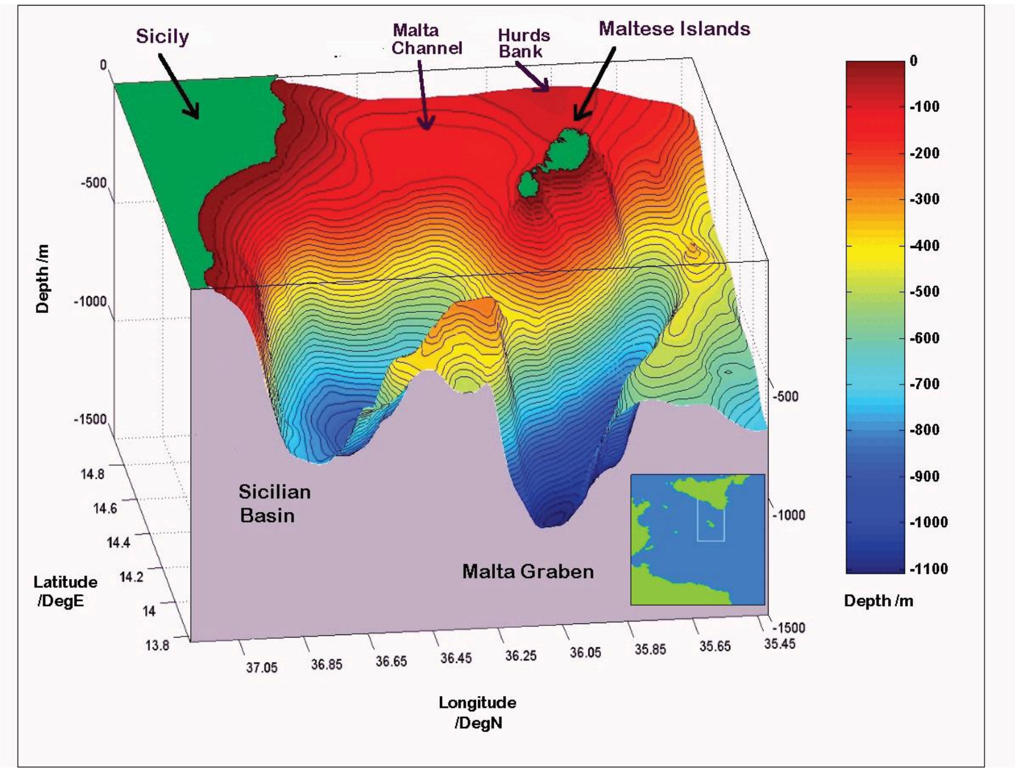


Fig. 2 : Three-dimensional view of the bathymetry around the Maltese Islands, based on the US Navy DBDB1 data set. (The insert shows the location of the model domain on the Malta shelf area) [27]

3.2. Life Baħar for Natura 2000

In order to protect threatened but ecologically important habitats and species, European Union Member States have adopted two sets of legislation, popularly known as the 'Habitats Directive' and the 'Birds Directive' for the creation of a system of protected sites known as the Natura 2000 network [28]. These sites are constituted of 'Special Protection Areas' for the protection of bird species, and 'Special Areas of Conservation' for the protection of vulnerable animals other than birds, as well as plants and habitats [28]. At present, they cover 18% of the EU's land and almost 6% of its seas, making Natura 2000 the largest coordinated conservation network worldwide [29]. All 28 European Union member states contribute to the network of Natura 2000 sites, and must ensure that the sites are managed in a sustainable manner to help the long-term survival of threatened species and habitats [29]. The designation of sites, both in terrestrial and marine areas, is based on the scientific assessment of each habitat type and the species present on the territory, which is evaluated by the European Commission [29].

Nonetheless, there is incomplete information concerning marine habitats and their biodiversity in Maltese waters, thus making it hard to designate appropriate marine areas for inclusion in the Natura 2000 network [30]. The Life Baħar for Natura 2000 is a research programme designed to investigate benthic ecosystems around Malta [30]. The aims of the Life Baħar project are to survey benthic marine habitats in the FMZ, focussing on sandbanks, reefs and marine caves using ROVs, Multibeam Echosounder and scuba diving, in order to complement and extend the already existing knowledge on these habitats, suggest new potential Natura 2000 sites, and increase awareness towards the need for conservation and management of marine resources [30]. It is a collaboration between the Maltese Environment and Resources Authority, the Maltese Government's Ministry for Sustainable Development, Environment and Climate Change, the University of Malta's Department of Biology, and the Fundación Oceana [30].

3.3. Aim of this study

The aim of this study is to assess the diversity of deep-sea echinoderms within the Malta 25-nautical miles Fisheries Management Zone, based on the ROV video footage obtained during two field campaigns in 2015 and 2016 as part of the Life Baħar for Natura 2000 project, and to describe the spatial and bathymetric distribution of the echinoderm fauna recorded during these surveys.

The specific objectives are:

1. the identification of every deep-water megabenthic echinoderm species found in the video data collected through ROV exploration;
2. the updating of the checklist of the currently known echinoderm fauna of the Maltese islands;
3. the estimation of the abundance and density, preferred habitat, associated bottom type and possible relation with habitat structuring species of the echinoderm species identified in the videos;
4. the analysis of the bathymetric distribution of every species found, and confirmation or updating of the known bathymetric limits of these species in the Mediterranean;
5. finally, the analysis of the spatial distribution of the dominant echinoderm species within the FMZ, by plotting maps of the density of the species in each surveyed zone.

Overall, this study should address the gap in knowledge on echinoderm species in Maltese deep waters.

II. Material and Methods

1. ROV sampling

The data used for this study were collected as part of the Life Baħar for Natura 2000 project in the summers of 2015 and 2016 by the research catamaran *Oceana Ranger* using a remotely operated vehicle (Saab Seaeye Falcon DR) [15]. The ROV has built-in high-definition photo and video cameras as well as position and depth tracking devices [15]. Because the advantage of the ROV is its ability to sample areas where trawl nets would rip, a majority of the dives were carried out over rocky substrata and steep escarpments to the south and south-west of the Maltese islands, along the edges of the Malta Graben as well as to the west, northwest and north of the island of Gozo. In 2015, 84 ROV dives were carried out between 1st June and 22 July, and in 2016, 88 dives were carried out between 27 May and 31 July. The dives situated in the eastern part of the FMZ however took place in waters shallower than 200m, and as this study focuses on deep waters, these were not analysed. In total, 138 dives were used for the present study. Their positions in the FMZ are represented in Fig. 3, and their GPS coordinates are compiled in Appendix 1.

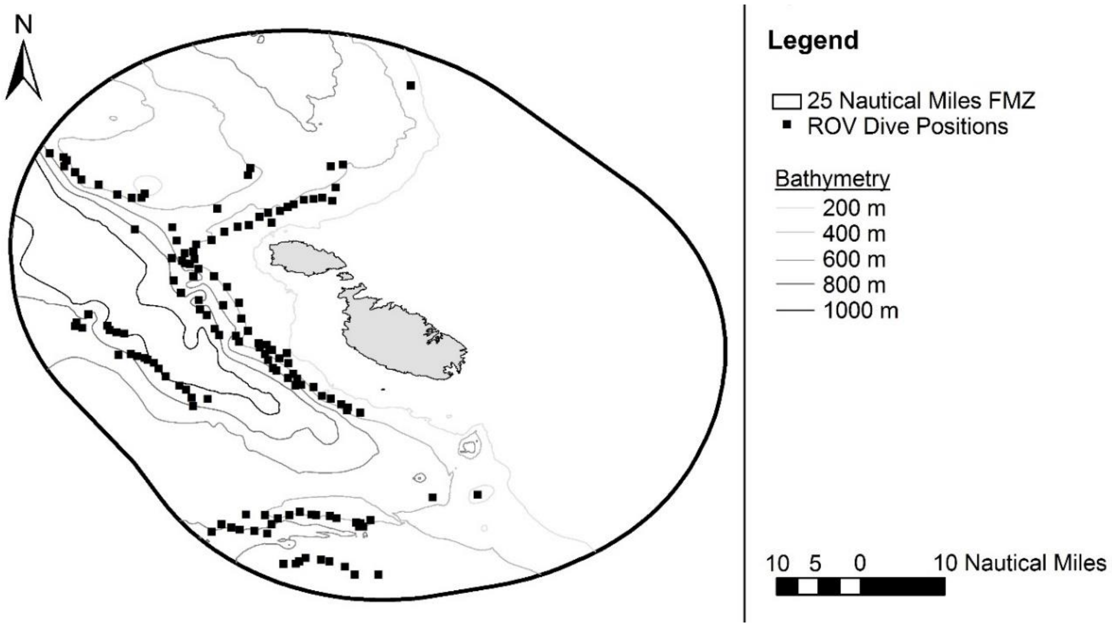


Fig. 3 : Positions of ROV dives made in 2015 and 2016 as part of the Life Baħar for Natura 2000 project and analysed in the present study. The oval around the Maltese Islands represents the boundary of the 25 nautical miles Fisheries Management Zone.

Knowing the average speed of the camera and the angle of view, it was estimated that the ROV filmed an area of approximately 650 m^2 every 60 minutes. From the total bottom time of each dive it could be inferred that the ROV covered a total area of 121,853 m^2 during the 138 dives analysed, and covered depths from 216m to 1030m. Fig. 4 was compiled to visualize the surface area that was explored in each 50m depth class. As shown in Fig. 4, the seafloor between 250m and 500m was the most explored, then dives between 500m and 950m were slightly less abundant, and finally the last two depth classes, between 950m and 1050m, were significantly less surveyed than the others.

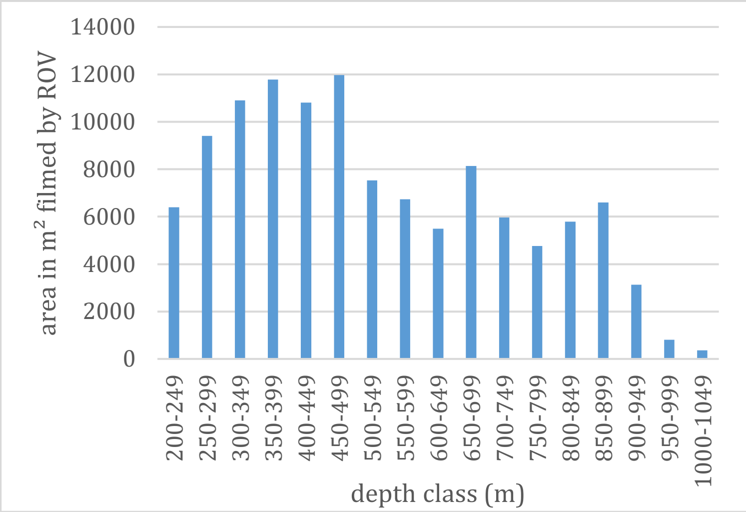


Fig. 4 : Surface area in m^2 covered by the ROV in the 138 dives analysed, classified by 50m depth strata.

Each dive was filmed in its entirety by the ROV in low resolution, however, in many cases, the image resolution was too low to correctly identify species. To address this, several clips in each dive were shot in high definition video, and these were used to assess the echinoderm diversity. In 80% of the dives, the duration of the HD footage was between 10% and 40% of the total bottom time. In the remaining 20%, the HD footage was shorter or longer than this. Nevertheless, these HD videos were taken every time a conspicuous or rare species was seen by the ROV operators, and every time an area rich in diversity was encountered. They are thus representative of the total species diversity of the whole dive, even if ultimately the total abundance is slightly underestimated. Because of this, one must keep in mind that the true abundance of echinoderms per area in the results is in fact larger than the estimates given here.

2. Video analysis

In total, 59 hours of HD footage were analysed. For each dive, the total bottom time was measured, as well as the duration of each HD video, and the dominant habitat type in each HD video was determined. For each echinoderm specimen encountered, a screenshot was taken and the dive station, date, GPS position, depth, microhabitat and habitat were recorded in an Excel matrix.

3. Identifications

Echinoderm species were identified to the lowest taxon possible on screenshots of ROV videos using keys and manuals, especially the reference “Echinodermata” by Enrico Tortonese [31]. Below are some additional notes on the criteria used to differentiate between similar species, with corresponding pictures of echinoderms found in the present study, for reference.

Crinoidea

There are two genera of crinoids in the Mediterranean: *Leptometra* and *Antedon*. The difference between them is based on the number of articles in the cirri: less than 30 in *Antedon* and more than 30 in *Leptometra*. There are two species of *Antedon*: *A.*

mediterranea and *A. bifida*. Again the difference is the number of articles in the cirri: 16 to 29 in *A. mediterranea* and not more than 18 in *A. bifida* [3][32][33].
Not every specimen could be identified through the number of cirri articles, but it was assumed that large aggregations of crinoids were monospecific.

Fig. 5: Stillshots from the ROV footage of crinoids identified as *Leptometra phalangium* (left) and *Antedon mediterranea* (right), with visible cirri.

Asteroidea

Odontaster mediterraneus and *Peltaster placenta* were differentiated based on 3 criteria; the number of marginal plates, the spines on the ventro-marginal plates and the number of joined terminal marginal plates. The genus *Ceramaster* which is superficially similar to these two species was also considered, but no specimen of *Ceramaster* were found in this study.

- *Odontaster mediterraneus*: odd number of marginal plates in the interradial area; conspicuous spines on ventro-marginals; first 2 or 3 terminal marginal plates joined along their mid-dorsal line.
- *Peltaster placenta:* even number of marginal plates; no conspicuous spines on ventro-marginals; only the first and perhaps second terminal marginal plates joined along their mid-dorsal line.
- *Ceramaster:* even number of marginal plates; no conspicuous spines on ventro-marginals; first three terminal marginal plates joined along their mid-dorsal line [31][32][33].

Fig. 6: Stillshots from the ROV footage of sea stars identified as *Peltaster placenta* (left) and *Odontaster mediterraneus* (right)

Sclerasterias richardi and *Sclerasterias neglecta* both have spines and proportionally longer arms than *P. placenta* or *O. mediterraneus*. The difference is that *S. neglecta* is larger (<160mm) than *S. richardi* (<30mm), has a reddish-brown colour and generally has 5 arms [21]. In contrast, *S. richardi* usually has 6 arms which can be unequal, for instance 3 long arms and 3 short ones [21].


Fig. 7: Stillshots from the ROV footage of sea stars identified as *Sclerasterias neglecta* (left) and *S. richardi* (right)

Echinoidea

Two species of Cidaridae, *Cidaris cidaris* and *Stylocidaris affinis*, are hard to distinguish from ROV images: *C. cidaris* has well defined terminal teeth on the large globiferous pedicellariae, and *S. affinis* does not [34]. However, the pedicellariae need to be examined using a microscope to be told apart. Thus, a different identification key (devised by Dr. Andreas Kroh, 20th November 2015 [22]) was used to differentiate them from pictures:

- *Cidaris cidaris*: Primary spines twice as long as corona diameter; corona and secondary spines white
- *Stylocidaris affinis*: Primary spines not much longer than corona diameter; corona and secondary spines reddish

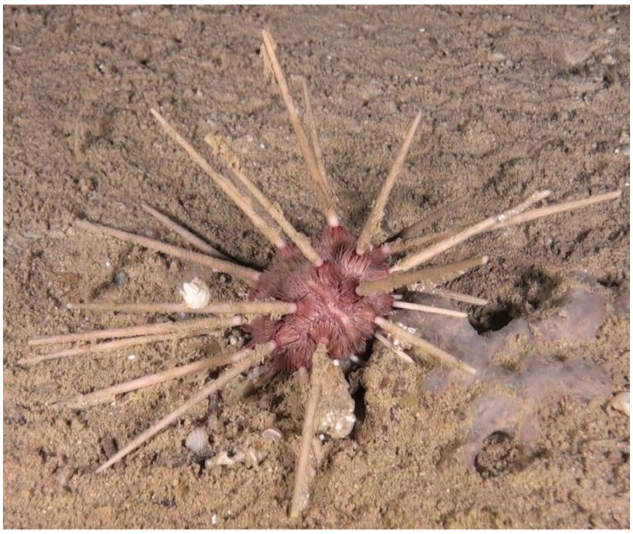



Fig. 8: Stillshots from the ROV footage of urchins identified as *Cidaris cidaris* (left) and *Stylocidaris affinis* (right)

Three species of *Gracilechinus* have been recorded in the Mediterranean Sea: *G. acutus, G. multidentatus* and *G. elegans* [16]. Seeing as the three species are impossible to correctly differentiate from images alone, *Gracilechinus* was only identified to the genus. *Echinus melo*, a closely related species, can be told apart with its rounder shape and shorter spines [31].

Fig. 9: Stillshots from the ROV footage of sea urchins identified as *Gracilechinus* sp. (left) and *Echinus melo* (right)

Photos of other echinoderms found in this study are provided in Appendix 2 for reference.

4. Habitat classification

Habitats were classified according to the revised list of Mediterranean and Black Sea habitats that was produced as part of the CoCoNet project [35]. A few adjustments were made to match the available information. For instance, muds, sands and a mixture of both are nearly impossible to distinguish in a video. As the granulometry was not measured with each ROV dive, the sediments were classified as "Bathyal sediment" with the corresponding dominant species if present. The full list of habitats used in the present study is given in Appendix 3.

Habitats were either used as in the list in Appendix 3, or if there were multiple habitats the following terminology was used:

- *Mosaic* (of 2 or 3 habitats present in equal proportions)
- Habitat 1 *with an enclave of* habitat 2, if habitat 1 was dominant.

Then, as it is sometimes difficult to separate a real white coral reef (Bathyal *Madrepora oculata* and/or *Lophelia pertusa* and/or *Desmphyllum dianthus* "reefs") from a coral colony settled on a rock (Bathyal rocks with *Madrepora oculata* and/or *Lophelia pertusa*), the following terminology was applied:

- if the coral colonies were very dense and no substratum was visible -> Reef
- if the space between two living colonies was larger than one colony, and there was dead coral in between -> Reef
- if the space between two colonies was larger than 1 colony, and there was rock in between -> Bathyal rocks with *Madrepora oculata* and/or *Lophelia pertusa*

The new categories added are:

- Bathyal rocks with Alcyonacea and Demospongiae
- Bathyal sediment with Desmospongiae and Foraminifera
- Bathyal sediment with Hydrozoa

See Appendix 3 for pictures of each case.

In the pie charts in the results, the major species are grouped into families or order for clarity, and only the most common habitat types for each species are displayed, the rest being grouped into “other rocks/sediment/mosaic”.

5. Microhabitat

For each echinoderm observed, the microhabitat was recorded as well as the general habitat type. The microhabitat was the substratum the individual was seen on, for instance: sediment, a rocky surface, inside a rock cavity, on anthropogenic substrata (sunken limestone slabs, glass bottles, metal containers, discarded fishing gear) or on other organisms. In the case of echinoderms using other organisms as a substratum, the most common corals were identified to the species level, the others were simply listed according to the phylum or class.

6. Bathymetric distribution

The studied area ranges from 216m to 1030m depth. The depths were sorted into 17 depth classes of 50m intervals. Before creating bathymetric distribution bar charts, the number of echinoderms found per depth class was standardised, by dividing it by the total area sampled at that depth class (Fig. 4).

7. Spatial distribution maps

Maps of the studied area showing the spatial distribution and density of the 15 most abundant echinoderms species were kindly created by Dr Leyla Knittweis-Mifsud of the University of Malta, using Geographical Information System (GIS) software. The density was calculated based on the total bottom time of each dive, knowing that the ROV covered 650 m^2 per 60 minutes.

III. Results

1. Total echinoderm diversity

In total, 9611 echinoderms belonging to 25 species (Table 1) were found in 59 hours of HD video footage analysed. The species comprised 11 asteroids, 2 crinoids, 4 ophiuroids, 5 echinoids and 3 holothurians. The most abundant species were in decreasing order *Antedon mediterranea*, *Leptometra phalangium*, *Stylocidaris affinis* and *Cidaris cidaris*, making up 92.83% of all observed echinoderms (Table 1).

Table 1: Checklist of echinoderms recorded in ROV videos within the 25 nautical miles FMZ around the Maltese Islands, with their observed depth range, mean density and frequency of occurrence. The relative abundance is the count of one particular species in relation to the total number of all individuals observed in the videos. The frequency of occurrence is the number of dives one species occurred in, divided by 138, the total amount of dives. * stands for new record in Maltese waters. Depths in bold extend the currently known depth range of the species.

Taxon	Count	Relative abundance (%)	Depth (m)	Mean density per 1000 m^2	Freq. of occurrence (%)
ASTEROIDEA					
Anseropoda placenta	17	0.18	332-501	0.124	7.2
Astropecten ?irregularis	1	0.01	341	0.008	0.7
Coronaster briareus	27	0.28	240-457	0.334	8.7
Hacelia attenuata	1	0.01	**266**	0.010	0.7
Hymenodiscus coronata	9	0.09	304-666	0.072	5.8
Luidia sarsii	1	0.01	271	0.005	0.7
Marginaster capreensis*	4	0.04	302-443	0.035	2.2
Odontaster mediterraneus	20	0.21	386-919	0.133	10.1
Peltaster placenta	123	1.28	241-**1020**	0.898	33.3
Sclerasterias neglecta*	20	0.21	287-**796**	0.229	8.7
Sclerasterias richardi	12	0.12	245-469	0.082	6.5
CRINOIDEA					
Antedon mediterranea	4347	45.23	239-390	62.229	7.2
Leptometra phalangium	2749	28.60	240-924	14.268	8.7
ECHINOIDEA					
Centrostephanus longispinus	1	0.01	**379**	0.013	0.7
Cidaris cidaris	692	7.20	222-1026	5.509	60.9
Echinus melo	5	0.05	241-876	0.048	3.6
***Gracilechinus* sp.**	116	1.21	256-1003	0.655	18.8
Stylocidaris affinis	1134	11.80	222-**1025**	9.132	70.3
HOLOTHUROIDEA					
***Holothuria* sp.**	5	0.05	239-250	0.110	2.2
Mesothuria intestinalis*	101	1.05	331-919	0.604	13.0
Parastichopus regalis	4	0.04	246-895	0.050	2.9
OPHIUROIDEA					
Astrospartus mediterraneus	3	0.03	**234-241**	0.026	1.4
***Ophiothrix* sp. (Grey morph)**	94	0.98	231-411	0.810	6.5
***Ophiothrix* sp. (Red morph)**	123	1.28	234-413	1.355	12.3
Ophiura ophiura	2	0.02	237-259	0.014	1.4

Stylocidaris affinis proved to be the most widely distributed species, found in 70.3% of all dives (Table 1). *C. cidaris* and *P. placenta* can also be considered common species, occurring in 60.9% and 33.3% of all dives respectively. Following those, the most frequent species were *Gracilechinus* sp., *Mesothuria intestinalis*, *Ophiothrix* sp. (red morph) and *Odontaster mediterraneus*. All other species were observed in less than 10% of dives.

1.1. Echinoderm diversity according to depth

In Fig. 10, the total number of species in each 50m depth class was standardised by dividing it by the total area covered at that depth class. It can be seen in Fig. 10 that the total echinoderm diversity is nearly constant from 200m to 950m, then seems to increase significantly in the last two depth strata, until 1050m depth.

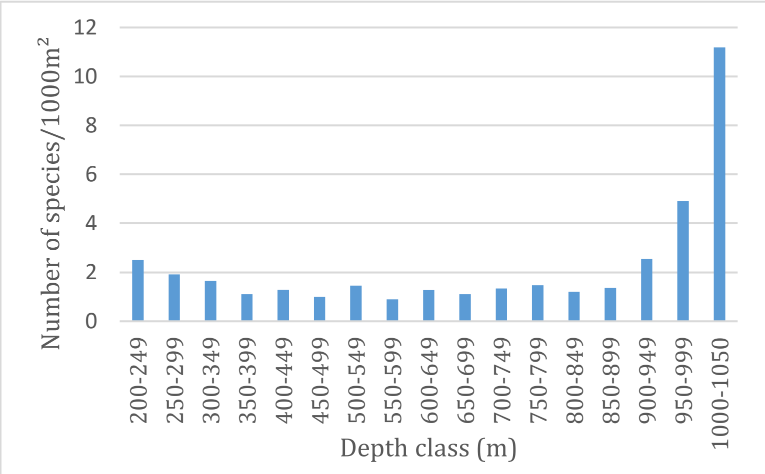


Fig. 10: Number of echinoderm species per 1000m^2 found in each 50m depth class

1.2. Update of the echinoderm fauna of the Maltese islands

The checklist of echinoderms of the Maltese islands by Tanti and Schembri [19] is currently the most recent list of Maltese echinoderms, although seven species have since been newly recorded; *Sclerasterias richardi* in 2008 [21], *Ophiotreta valenciennesi rufescens*, *Odontaster mediterraneus* and *Luidia sarsii* in 2009 [20], *Hymenodiscus coronata* and *Ophiothrix quinquemaculata* in 2015 [18] and *Coronaster briareus* in 2016 [15].

In the present study, 4 specimens of *Marginaster capreensis* (Gasco, 1876), 101 specimens of *Mesothuria intestinalis* (Ascanius, 1805) and 20 specimens of *Sclerasterias neglecta* (Perrier, 1891) were recorded for the first time in Maltese waters. Thus, the checklist of echinoderms in Malta can be updated with these three species (see Appendix 4).

1.3. Update of the depth range of Mediterranean echinoderms

The present study allowed to record the depth of occurrence of each echinoderm specimen, and six species were observed at deeper depths than their currently known limit (in bold in Table 1).

Asteroidea

In the present study, one individual of *Hacelia attenuata* was found at a depth of 266m, which is deeper than the range of 1-190m given in the checklist of Mediterranean echinoderms in Coll et al. [11]. *Peltaster placenta* is reportedly found down to depths of 500m [11]. More recently, it was observed down to 678m depth in the MEDITS trawl surveys held in Maltese waters [18]. However, in the present study, 60 individuals of this species (49% of all *P. placenta* observed) were recorded deeper than 500m, and 38 were deeper than 700m, down to a maximum of 1020m. Similarly, *Sclerasterias neglecta* is reported by Coll et al.[11] to live down to 485m, but in the present study it was observed down to 796m, with 4 individuals found deeper than 500m.

Ophiuroidea

Three specimens of *Astrospartus mediterraneus* were recorded at 234m and twice at 241m, although the currently known depth range for this species is only 50-188m [11].

Echinoidea

Centrostephanus longispinus was found in this study at 379m depth, although Coll et al. report a depth range of 40-363m [11].

Finally, *Stylocidaris affinis* is reported down to 1000m depth [11], but was observed here down to 1025m depth.

All other echinoderm species found in the present study fall within their currently known depth range.

1.4. Bottom type and habitat classification

Fig. 11 below shows the proportion of different bottom types surveyed by the ROV. This information is useful for comparing the preferred bottom types of each species. If a species occurs on the general bottom types (rocky, sedimentary, mosaic or reef) in a similar proportion as the general distribution of bottom types in Fig. 11, this will suggest that the species has no preference for any particular bottom type and can be found on almost any type of bottom. The results on the bottom preferences of individual classes will be given in the sections below.

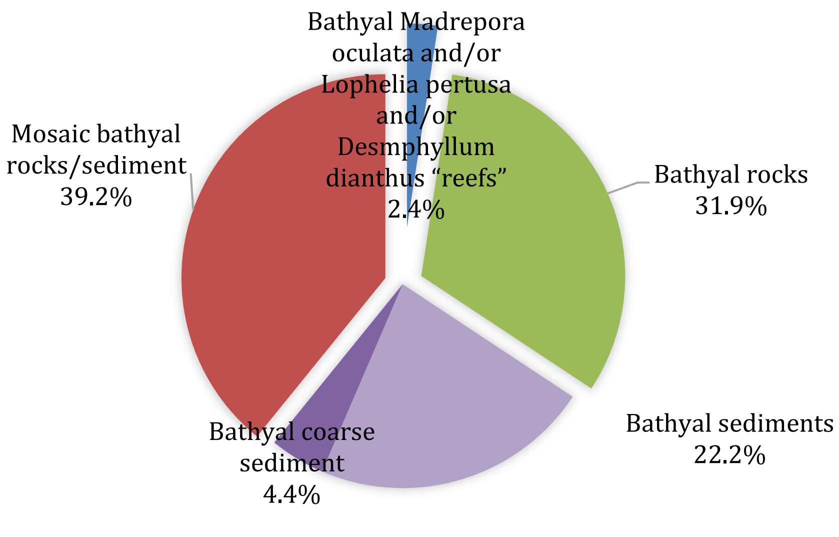


Fig. 11: Percentage of general bottom types in whole area surveyed by ROV in 2015 and 2016

2. Spatial and bathymetric distribution, habitat description per class and species of echinoderms

2.1. Echinoidea

Cidaridae

Sea urchins belonging to the family Cidaridae, *Stylocidaris affinis* and *Cidaris cidaris*, were the most widely distributed echinoderms in waters deeper than 200m around Malta (Table 1).

Bathymetric distribution

As Fig. 12 shows, *Stylocidaris affinis* was generally more abundant than *Cidaris cidaris*. The two cidarids were distributed differently: *S. affinis* was more common in shallower waters, then its abundance decreased slightly towards deeper waters. *C. cidaris* showed a less clear distribution pattern; it was most common between 400m and 500m, and then between 850m and 900m, and again between 250m and 300m. Seeing as how both species are found in the shallowest and deepest depth classes considered, it is likely that their actual depth range exceeds the depth limits of this study (200m and 1050m).

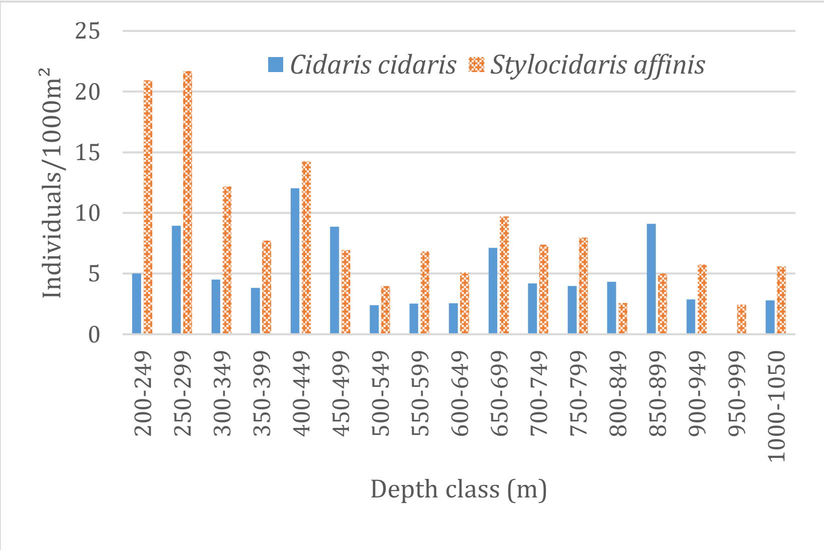


Fig. 12: Bathymetric distribution of *Cidaris cidaris* and *Stylocidaris affinis*

Microhabitat

Fig. 13 shows that both species of cidarids were mostly found on a rocky substratum, followed by sediments, then on corals. They were also recorded, at a lower frequency, on anthropogenic objects such as sunken limestone slabs, metal containers and ropes, and on organisms other than corals, for example, on sponges. *C. cidaris* was more frequently observed on coarse sediment, on anthropogenic objects, on corals and other basibiota than *S. affinis*, which was in 90.6% of cases found on rocks or on fine sediment. Neither species appeared to be specialised for one particular microhabitat.

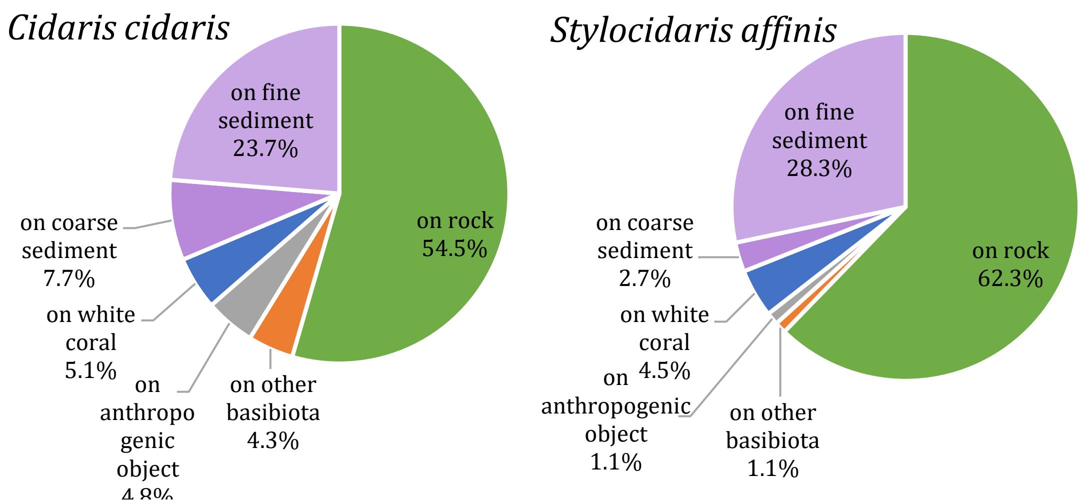


Fig. 13: Microhabitats of *Cidaris cidaris* and *Stylocidaris affinis*

Habitat type

When compared to Fig. 11, both species were distributed on the different bottom types in the same proportions as the occurrence of these bottom types in the area surveyed (Fig. 14 and 15). This shows that neither *C. cidaris* nor *S. affinis* have a preference for any one particular bottom type. However, there were some differences between the species; *S. affinis* seems to be more often associated with black coral (Antipatharia) than *C. cidaris*, while *C. cidaris* was more frequently found together with crinoids and Alcyonacea such as *Callogorgia verticillata* than *S. affinis.*

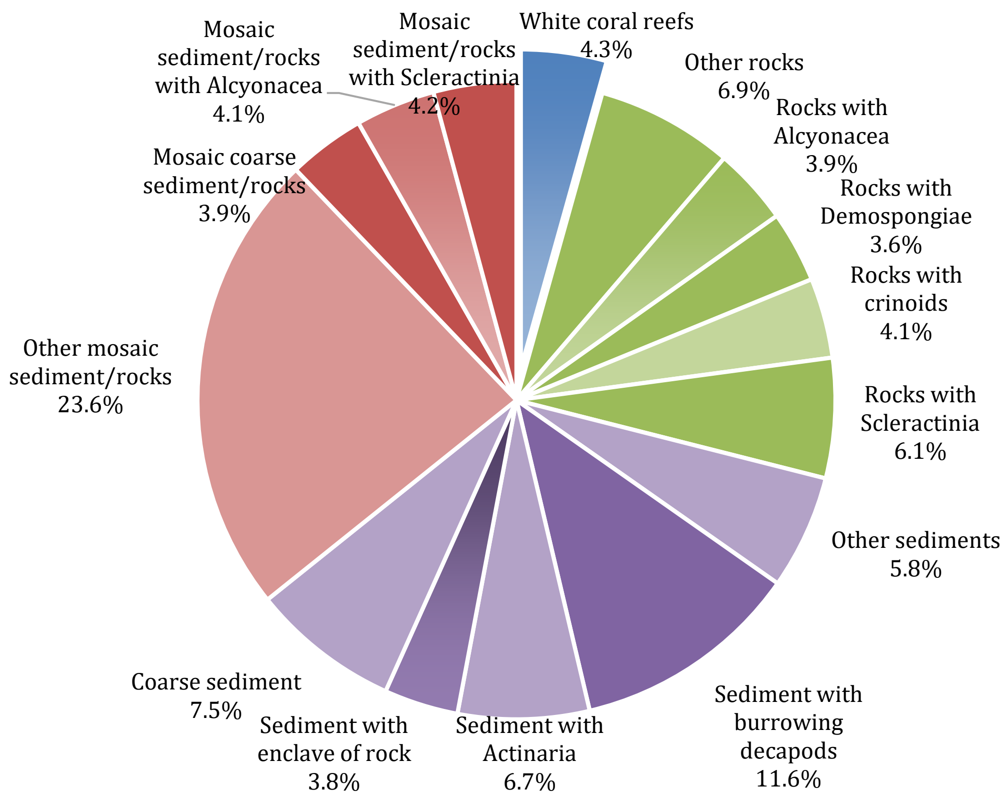


Fig. 14: Preferred habitat types of *Cidaris cidaris*

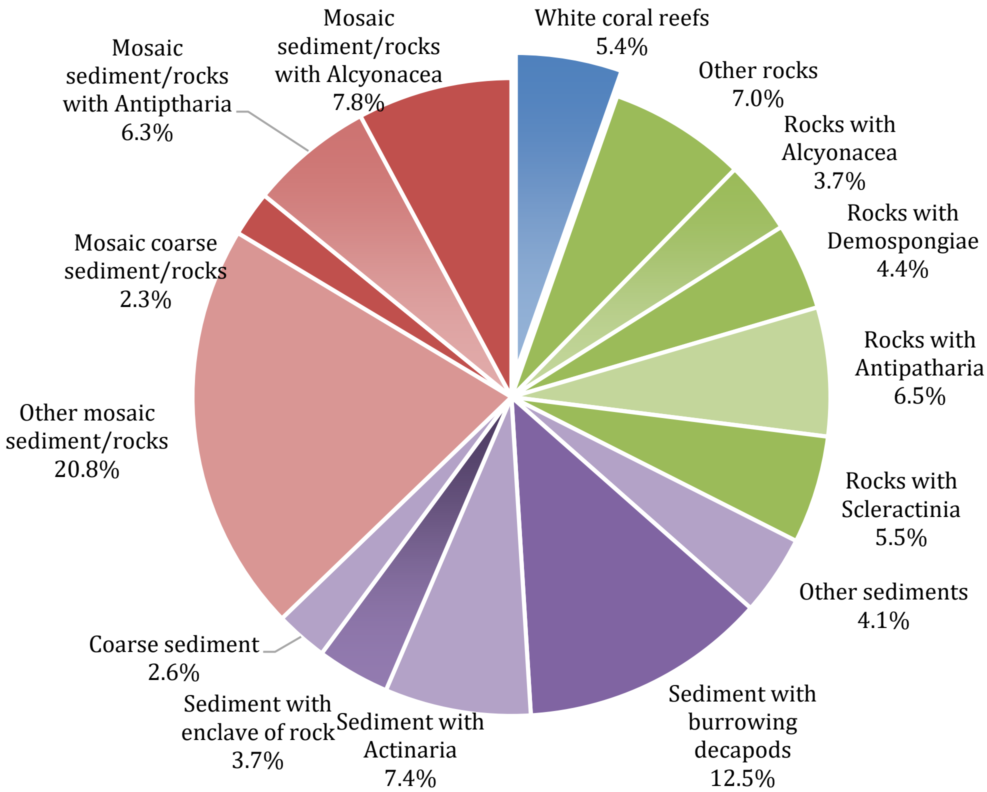


Fig. 15: Preferred habitat types of *Stylocidaris affinis*

Spatial distribution

As can be seen in Fig. 16, both Cidaridae species were distributed widely across the surveyed area. *C. cidaris* and *S. affinis* were present in 60.9% and 70.3% of all dives respectively. However, the density of individuals differs: *S. affinis* occurred in densities up to 120 individuals/1000m^2, but *C. cidaris* only occurred at maximum densities of 60 individuals/1000m^2. When comparing the maps, note that the same size circles represent twice as many individuals in Fig. 16b than in Fig. 16a.

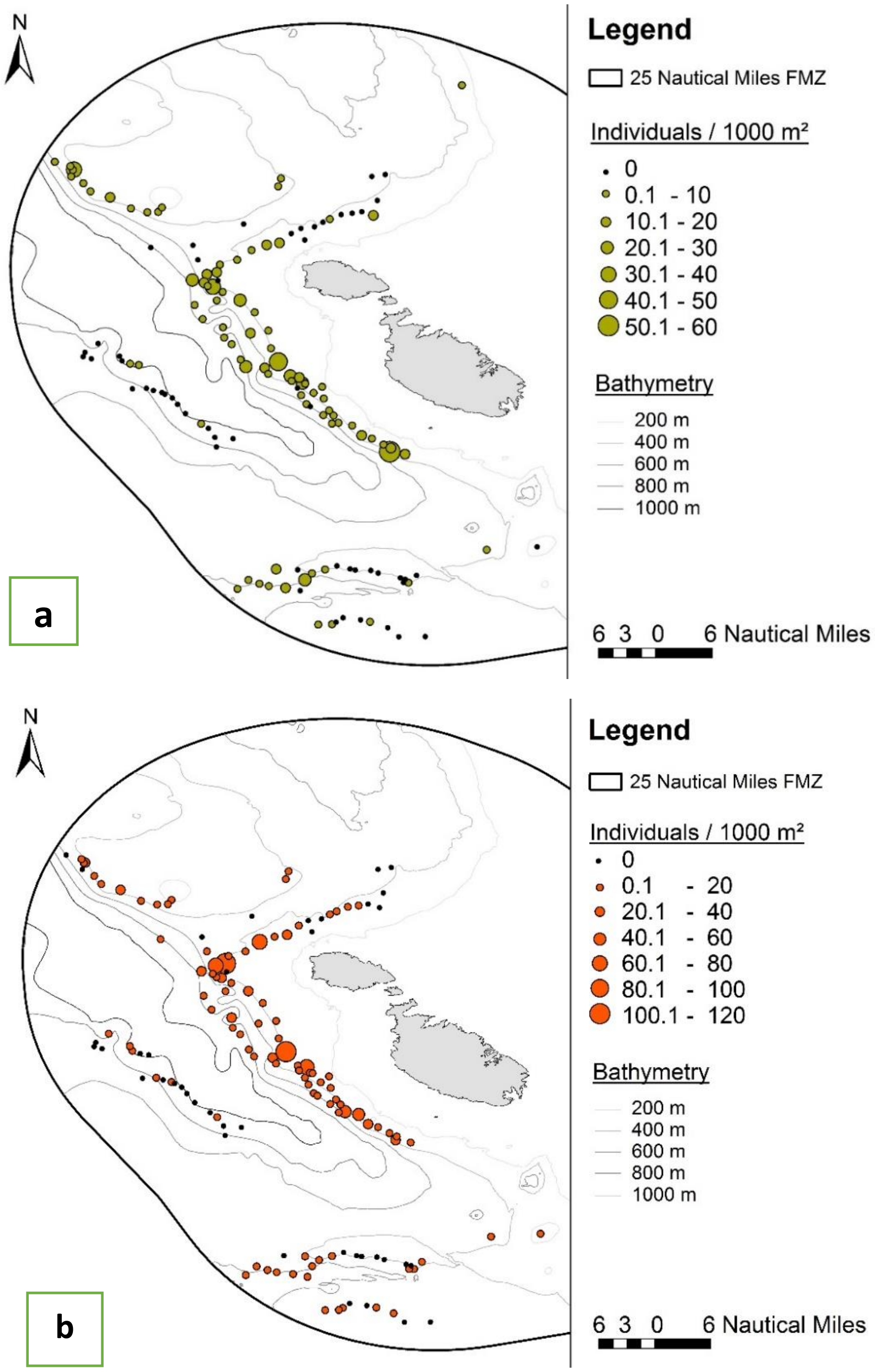


Fig. 16: Maps of Malta showing the spatial distribution and density of *Cidaris cidaris* (a) and *Stylocidaris affinis* (b) individuals recorded through ROV dives within the 25 nautical miles FMZ.

***Gracilechinus* sp.**

Bathymetric distribution

Individuals of *Gracilechinus* sp. were found from 256m down to 1003m depth, but showed a clear predominance in deeper waters. Seeing the distribution in Fig. 17, the depth range of *Gracilechinus* sp. almost certainly extends beyond 1003m.

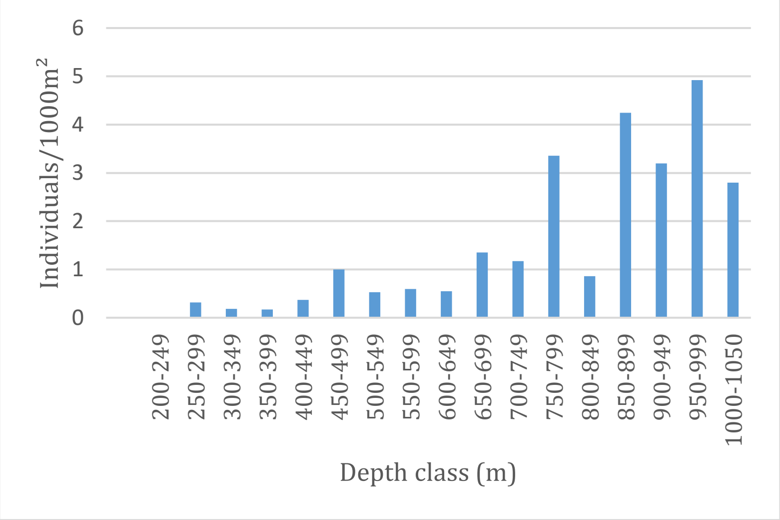


Fig. 17: bathymetric distribution of *Gracilechinus* sp.

Microhabitat and habitat type

Gracilechinus sp. was mostly found on rocks, but 44% were attached to colonies of corals, in most cases white corals such as *Madrepora oculata* and *Lophelia pertusa,* but also on gorgonians such as *Callogorgia verticillata* (Fig.18). From Fig. 19, compared to Fig. 11, it can be inferred that *Gracilechinus* sp. is commonly found on coral reefs and in rocky habitats dominated by scleractinians, black corals or gorgonians. It was only rarely observed on sedimentary bottoms (Fig. 18 and Fig. 19).

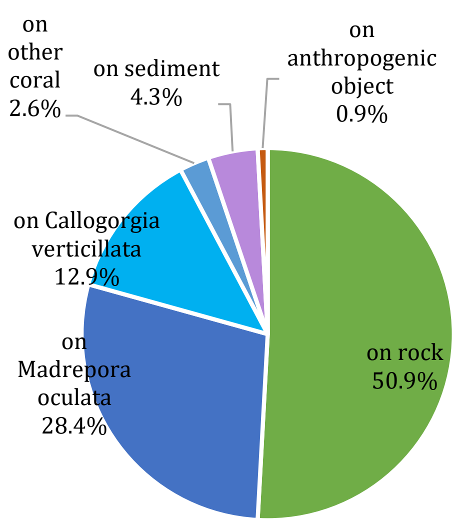


Fig. 18: Microhabitat of *Gracilechinus* sp.

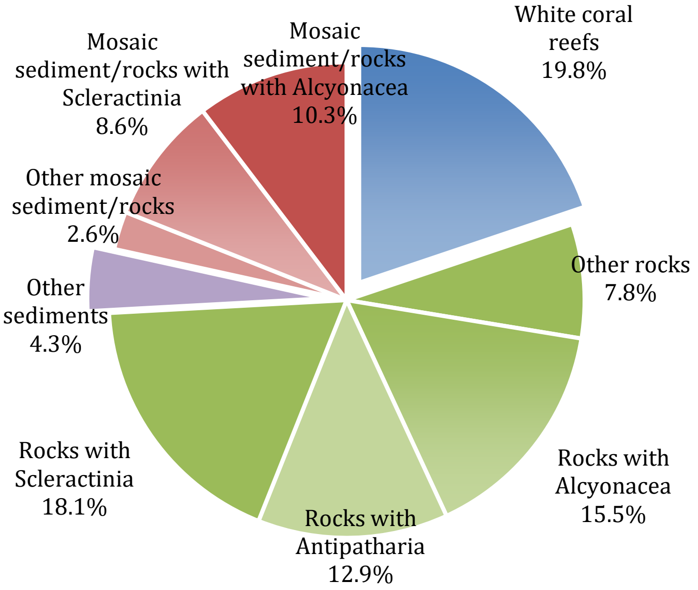


Fig. 19: Preferred habitat types of *Gracilechinus* sp.

Spatial distribution

Fig. 20 shows that *Gracilechinus* sp. seems to be distributed over the whole studied area, it was not confined to one particular sector; however, it was more frequent along the escarpment on the east side of the Malta Graben, with a maximum mean density of 15.5 individuals/1000m^2, which is less than for both cidarids. As it occurred in 18.7% of all dives (Table 1), it was relatively common in the studied area.

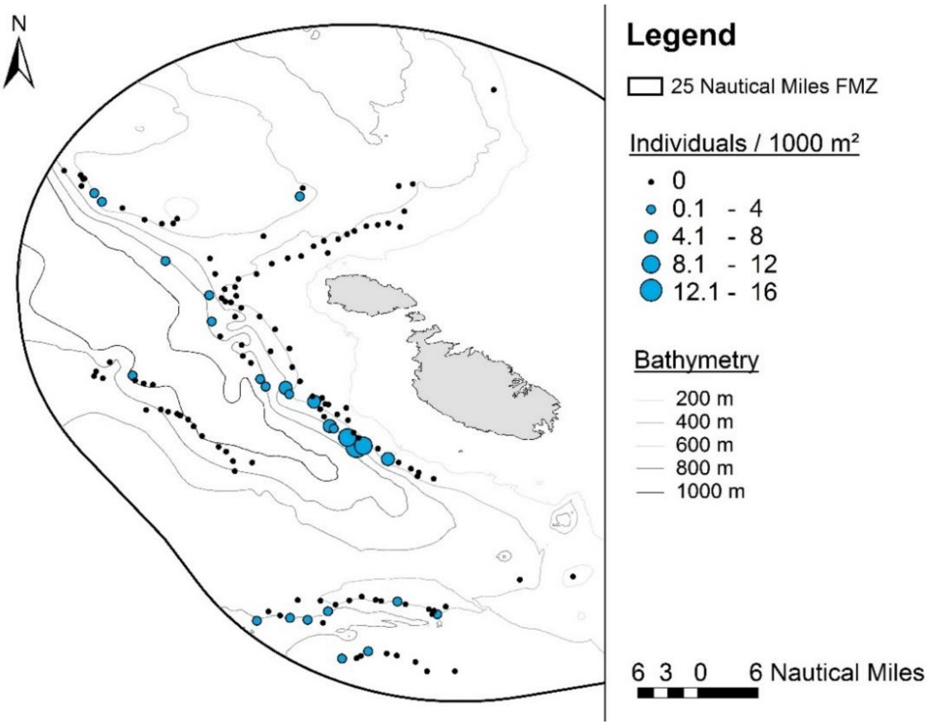


Fig. 20: Map of Malta showing the spatial distribution and density of *Gracilechinus* sp. individuals recorded through ROV dives within the 25 nautical miles FMZ.

Other echinoids

One specimen of *Centrostephanus longispinus* was found at 379m depth, on sediment but in an area constituted of a mosaic of rocks and sediment. Five specimens of *Echinus melo* were seen between 241m and 876m, either on rocks or attached to corals, generally in rocky habitats. No irregular urchins were found.

2.2. Crinoidea

Two species of crinoids are present in Maltese waters, *Antedon mediterranea* and *Leptometra phalangium*, and both occur in very dense aggregations, with mean densities of 62.2 *A. mediterranea*/1000m^2 and 14.3 *L. phalangium*/1000m^2 (Table 1).

Bathymetric distribution

Almost all (99.6%) of the *A. mediterranea* individuals were found between 200m and 300m; the remaining 0.4% were found down to 400m depth. As for *Leptometra phalangium,* 99.6% were found between 400m and 600m, one individual was seen at 240m, and nine individuals between 800m and 950m. They show a clear preference for particular depth ranges, with *A. mediterranea* occurring in shallower water than *L. phalangium*. According to Fig. 21, *A. mediterranea* is likely to be found shallower than 200m.

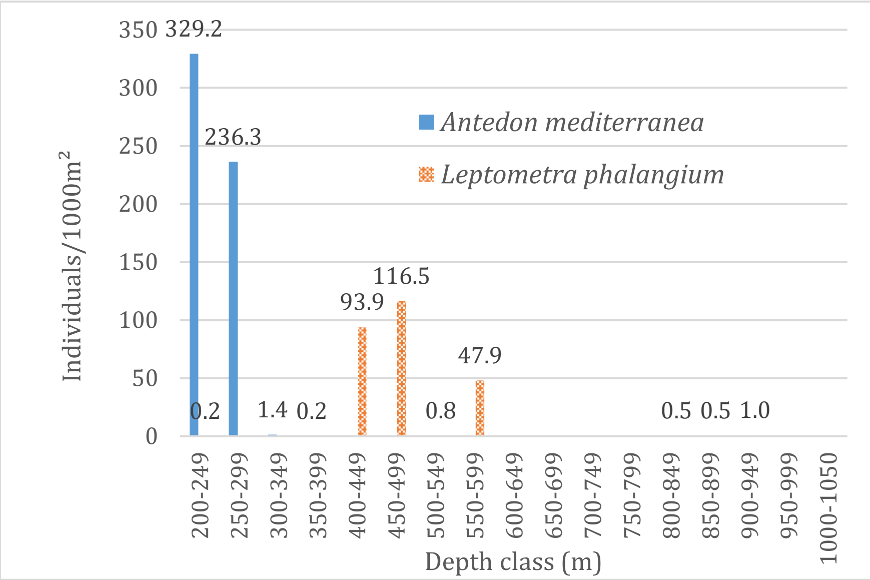


Fig. 21: Bathymetric distribution of *Antedon mediterranea* and *Leptometra phalangium*

Microhabitat

Both crinoid species were usually found attached to a rocky substratum, sometimes on anthropogenic objects such as discarded fishing gear, and very rarely, on sediment (Fig. 22). 7.1% of all *L. phalangium* were found attached to *Madrepora oculata* and 0.3% on *Callogorgia verticillata* and on barnacles.

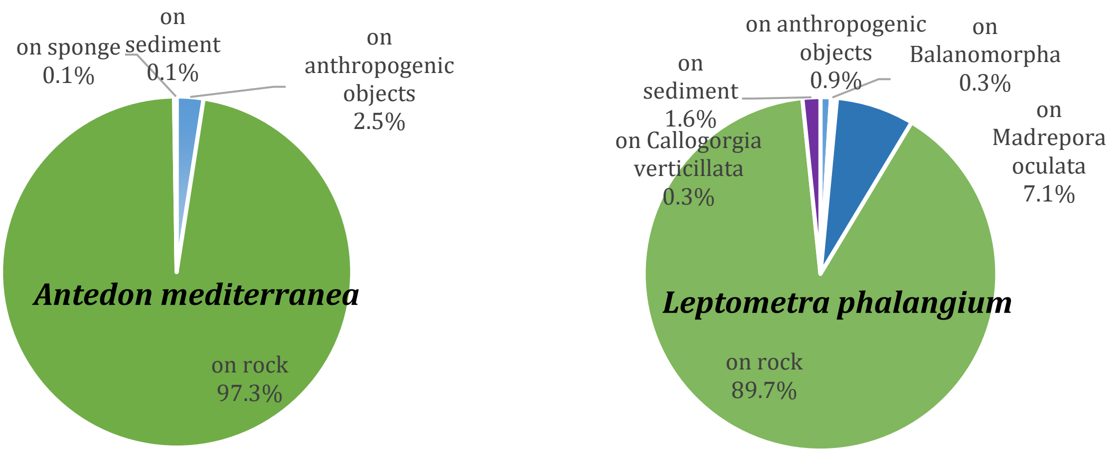


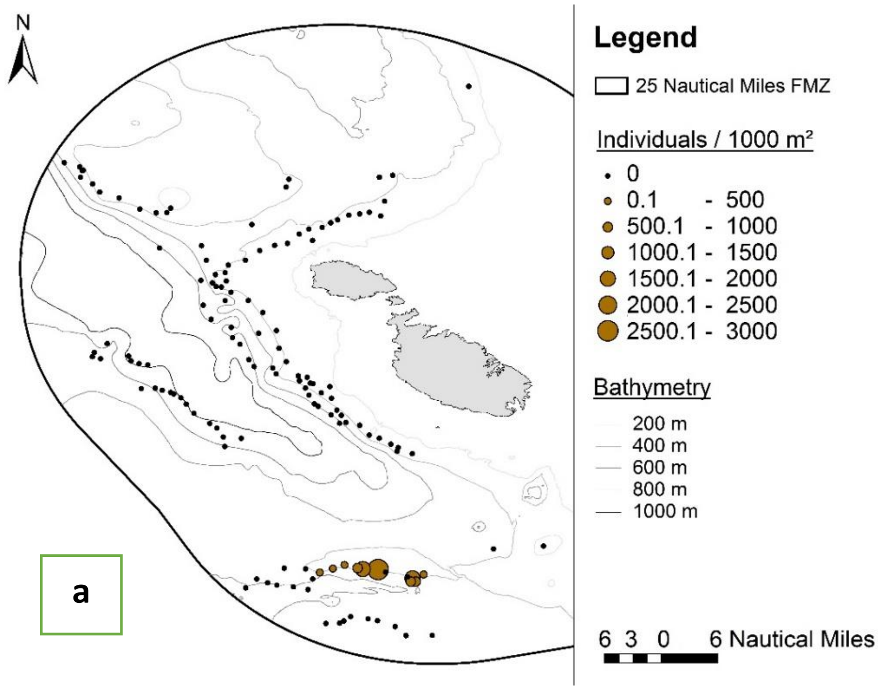
Fig. 22: Microhabitats of *Antedon mediterranea* and *Leptometra phalangium*

Habitat type

Because of their dense aggregations, crinoids were most often the dominant species in the habitat, and were almost exclusively, in 93% of cases for *L. phalangium*, 97% for *A. mediterranea*, found in habitats labelled "Bathyal rocks with *Leptometra phalangium* and/or *Antedon mediterranea*" and "Mosaic bathyal sediment/rocks with *Leptometra phalangium* and/or *Antedon mediterranea*".

Spatial distribution

Crinoids appear to be distributed much more sparsely than other echinoderms. *A. mediterranea* occurred in 7.2% of all dives, but only in a single area to the south of the Maltese islands (Fig. 23a). However, it can occur in very dense aggregations of up to 2937 individuals/1000m^2. *L. phalangium* occurred in one large aggregation of 1588 individuals/1000m^2, also to the south of Malta (Fig. 23b), but in deeper water than *A. mediterranea*. It also occurred in smaller densities, between 182 individuals/1000m^2 and 1 individual/1000m^2, in 8.6% of all dives, in various places in the FMZ but generally deeper than *A. mediterranea*.



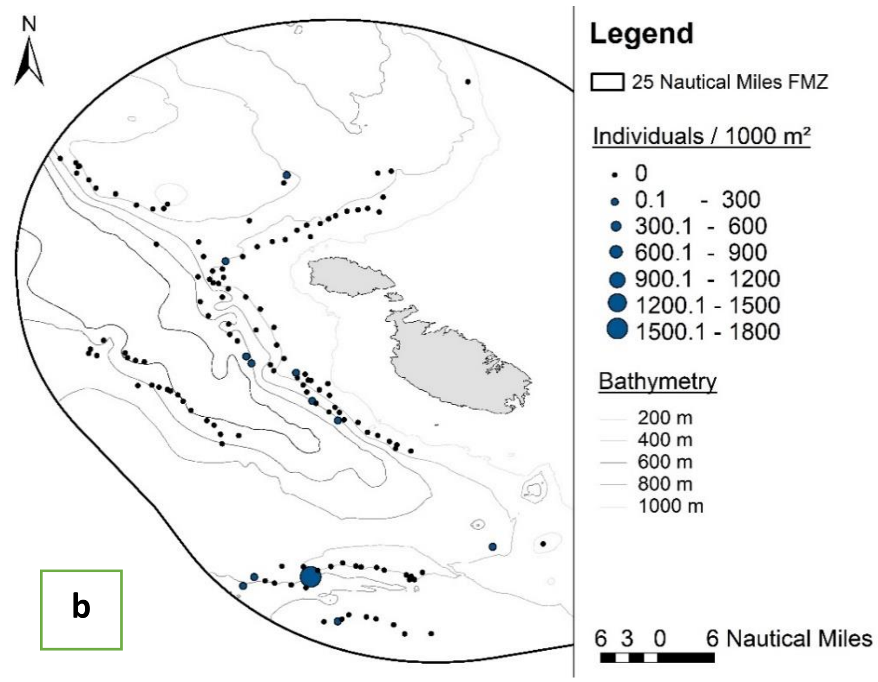


Fig. 23: Maps of Malta showing the spatial distribution and density of *Antedon mediterranea* (a) and *Leptometra phalangium* (b) individuals recorded through ROV dives within the 25 nautical miles FMZ.

2.3. Asteroidea

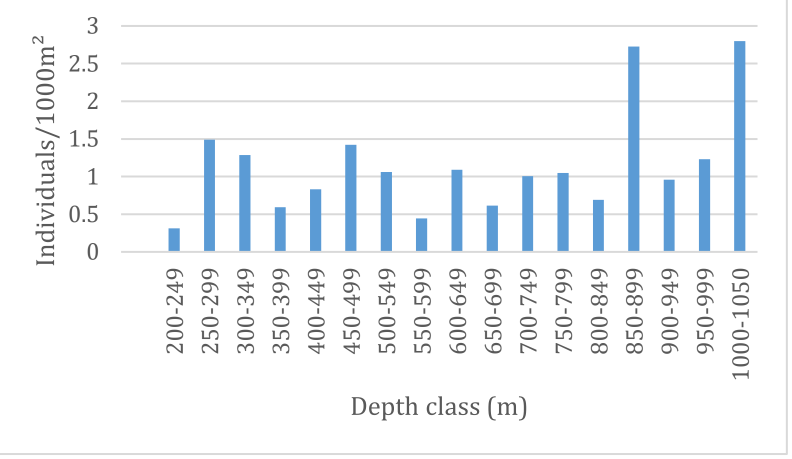
2.3.1. Valvatacea

Peltaster placenta

Bathymetric distribution

As seen in Fig. 24., *Peltaster placenta* seems to be distributed across all surveyed depth zones at different densities, perhaps more abundant towards the deeper areas.

Fig. 24: Bathymetric distribution of *Peltaster placenta*



Microhabitat

A large majority of *P. peltaster* were on rocky substrata (87.8%), the rest were found on sediment (7.3%), on coral (4.1%), and entangled in a fishing net (0.8%).

Habitat type

P. placenta had a clear preference for rocky habitats (Fig. 25, compared to Fig.11), mostly where scleractinians, black corals or gorgonians were present, but also in areas with less species diversity. It was very rare on sedimentary bottoms.

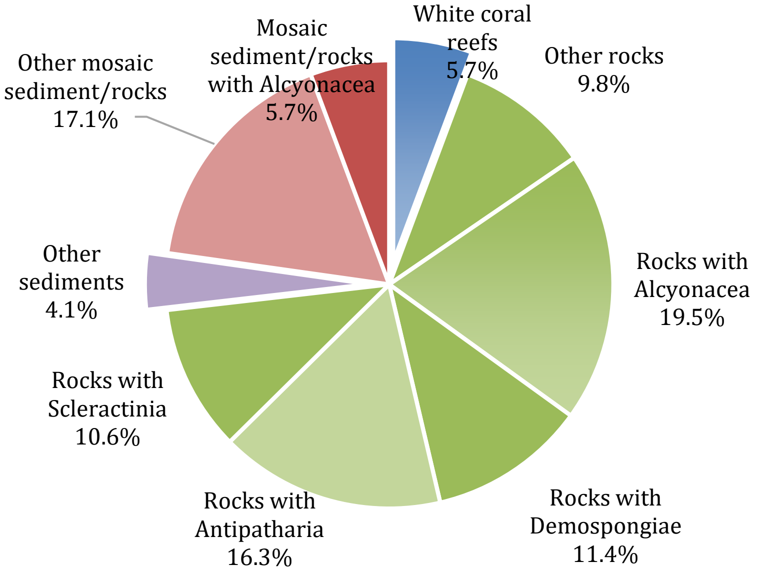


Fig. 25: Preferred habitat type for *Peltaster placenta*

Spatial distribution

P. placenta was quite a common and widely distributed species in Maltese waters, occurring in 33.3% of all dives (Table 1). It was more predominant along the escarpments of the Malta Graben (Fig. 26). It did not occur in large groups but always individually, with the largest density in a dive being 11 individuals/1000m^2.

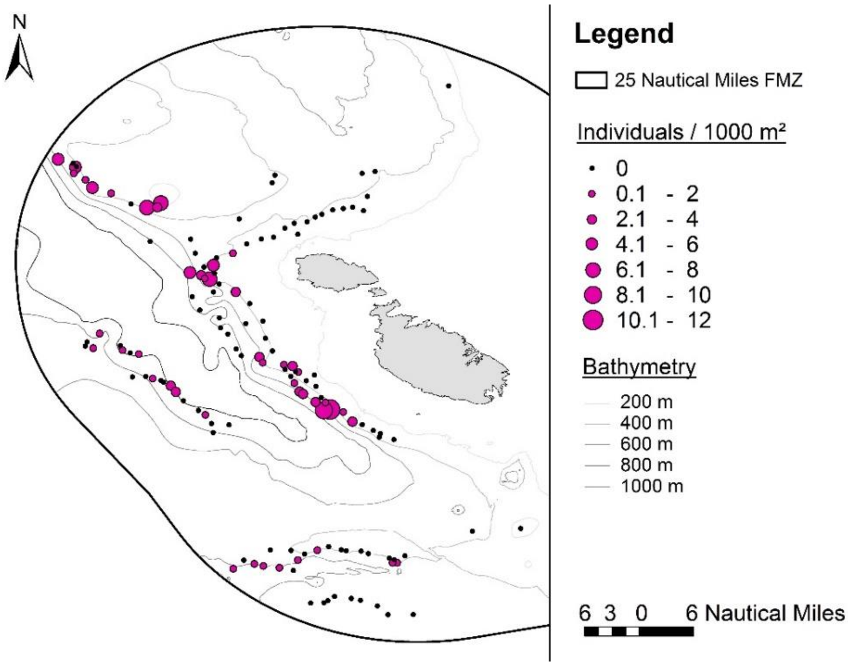


Fig. 26: Map of Malta showing the spatial distribution and density of *Peltaster placenta* individuals recorded through ROV dives within the 25 nautical miles FMZ.

Odontaster mediterraneus

Bathymetric distribution

Odontaster mediterraneus seems to be present over a large depth range, with many gaps in the bathymetric distribution because of the small number of specimens found (20). It was more predominant in deeper waters, especially between 800m and 850m (Fig. 27).

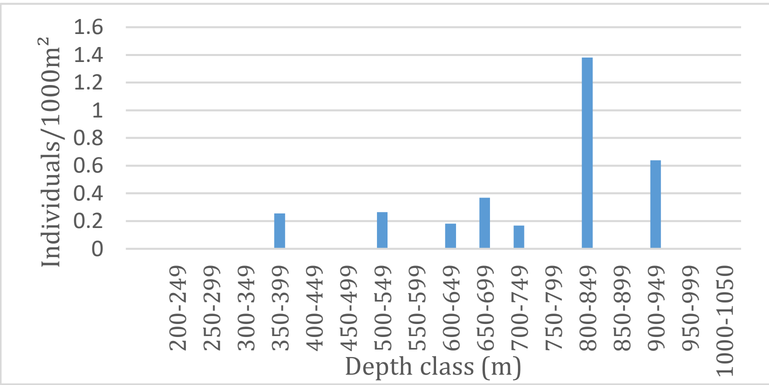


Fig. 27: Bathymetric distribution of *Odontaster mediterraneus*

Microhabitat and habitat type

O. mediterraneus was exclusively found on rocky surfaces, mostly in rocky habitats with various dominating species, but also on bottoms consisting of a mosaic of rocks and sediment.

Spatial distribution

In this study, *O. mediterraneus* was rarer than *P. placenta*, but likewise occurring as isolated individuals and in the same general areas as *P. placenta*. The only exception is the north-west of the FMZ, where *P. placenta* was frequent but no *O. mediterraneus* were found.

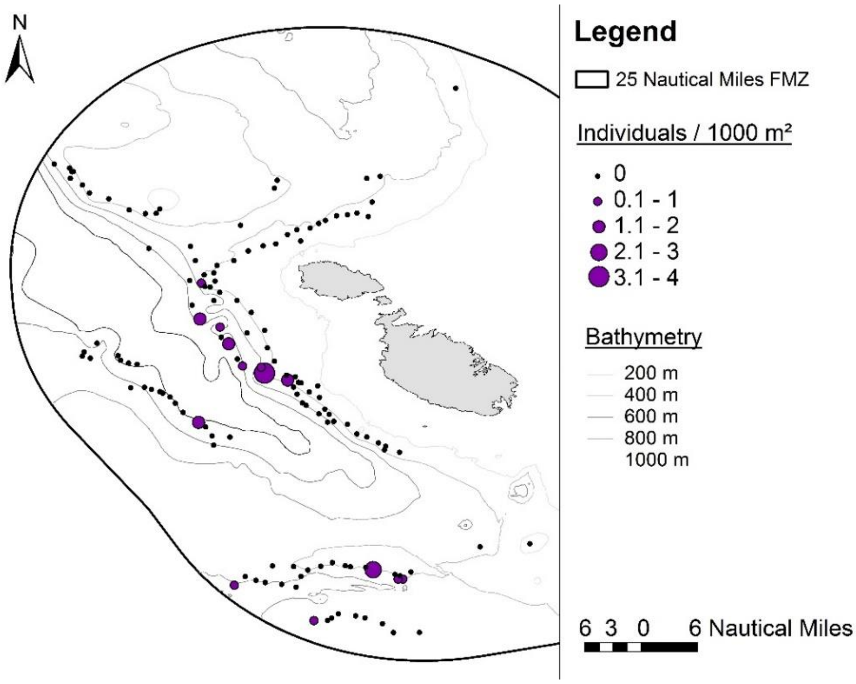


Fig. 28: Map of Malta showing the spatial distribution and density of *Odontaster mediterraneus* individuals recorded through ROV dives within the 25 nautical miles FMZ.

Anseropoda placenta

Bathymetric distribution

17 individuals of *A. placenta* were recorded within a limited depth range, between 332m and 501m, with a greater abundance towards the deeper end of this depth range, between 450m and 500m (Fig. 29).

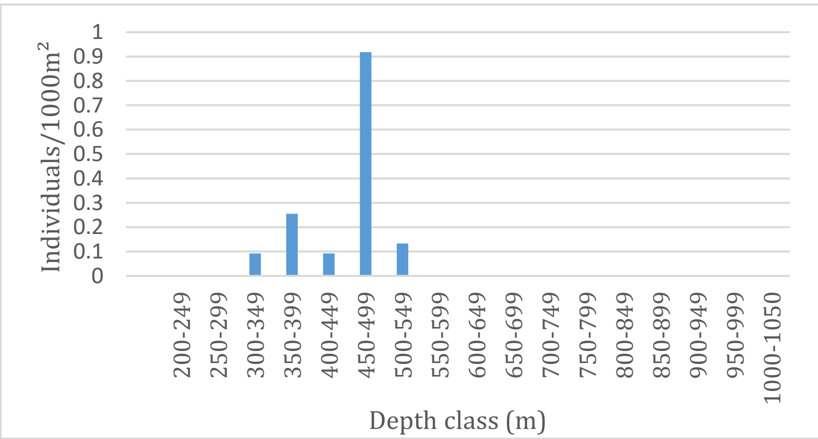


Fig. 29: Bathymetric distribution of *Anseropoda placenta*

Microhabitat and habitat type

A. placenta was seen exclusively on sedimentary surfaces, in sedimentary habitats dominated by burrowing decapods or on bottoms consisting of mosaics of rocks and sediment.

Spatial distribution

A. placenta was not a very common species; it occurred in 7,2% of dives, only in the south and north-west parts of the FMZ, and never around the Malta Graben to the west of the Maltese islands. It always occurred individually, with a maximum density of 3.5 individuals/1000m^2.

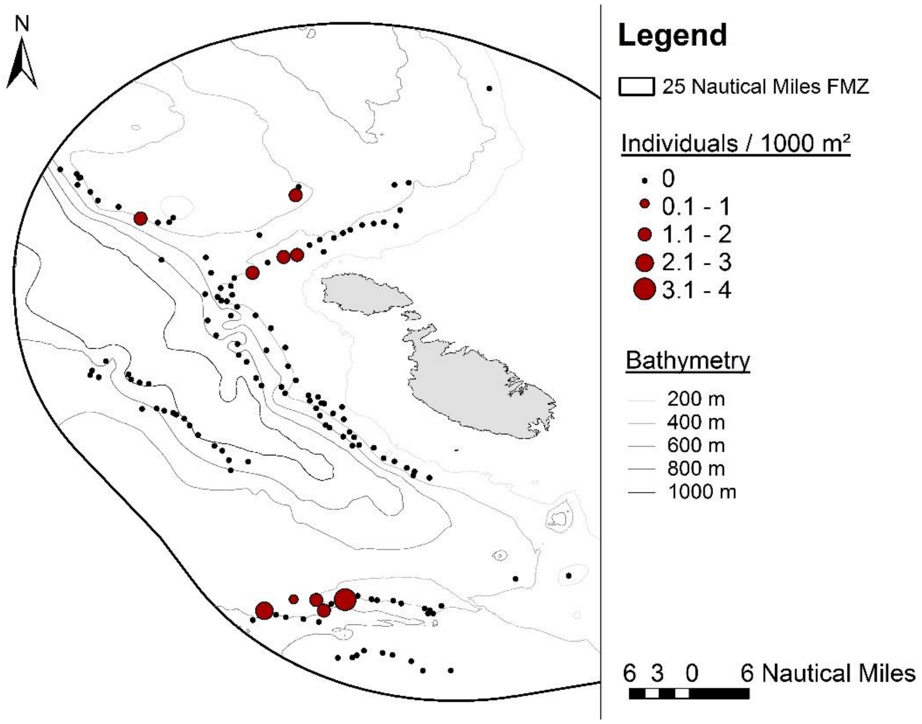


Fig. 30: Map of Malta showing the spatial distribution and density of *Anseropoda placenta* individuals recorded through ROV dives within the 25 nautical miles FMZ.

Other Valvatacea

Four specimens of *Marginaster capreensis* were found for the first time in Malta, at depths from 302m to 443m. They were always positioned on rocks, in mixed habitats of rocks and sediment.

One specimen of *Luidia sarsii* was found at 271m depth, on loose sediment dominated by burrowing decapods.

One specimen of *Hacelia attenuata* was seen at 266m depth, on a rocky substratum within a mosaic of sediment and rocks dominated by Alcyonacea.

An individual of *Astropecten* (possibly *A. irregularis*) was seen at 341m depth on loose sediment dominated by burrowing decapods.

2.3.2. Forcipulatacea

Coronaster briareus

Bathymetric distribution

Coronaster briareus seems to have a normal distribution in shallower waters (Fig. 31), with a preference for the 300-349m depth zone and has a declining abundance above and below this zone. From the bathymetric distribution pattern, it is likely to also be found shallower than 200m depth.

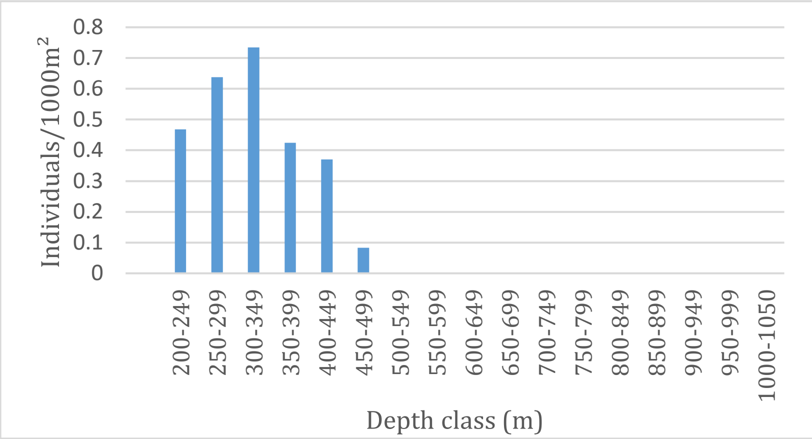
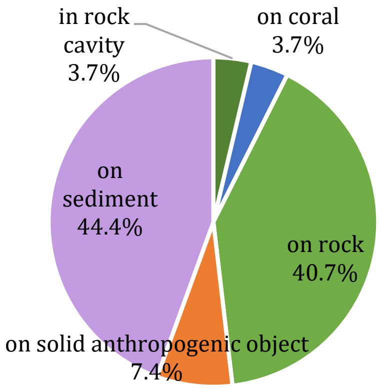


Fig. 31: Bathymetric distribution of *Coronaster briareus*.

Microhabitat

Coronaster briareus does not seem to have a preference for a loose or hard substratum since it was found frequently on both types (Fig. 32). It was also found occasionally on anthropogenic objects such as limestone slabs and a metal container, in a rock cavity and on a soft coral.

Fig. 32: Microhabitats of *Coronaster briareus*



Habitat type

48.1% of *C. briareus* individuals were recorded on sediments dominated by decapods, 29.6% were associated with rocky habitats dominated by crinoids, and the rest were found in various other rocky habitats.

Spatial distribution

The majority (26 out of 27 specimens) of *C. briareus* were observed in a relatively small area at the South of Malta (Fig. 33), the same area in which all the *Antedon mediterranea* specimens were found. Only one of 27 *C. briareus* was recorded outside of this area, and occurred to the north-west of Gozo.

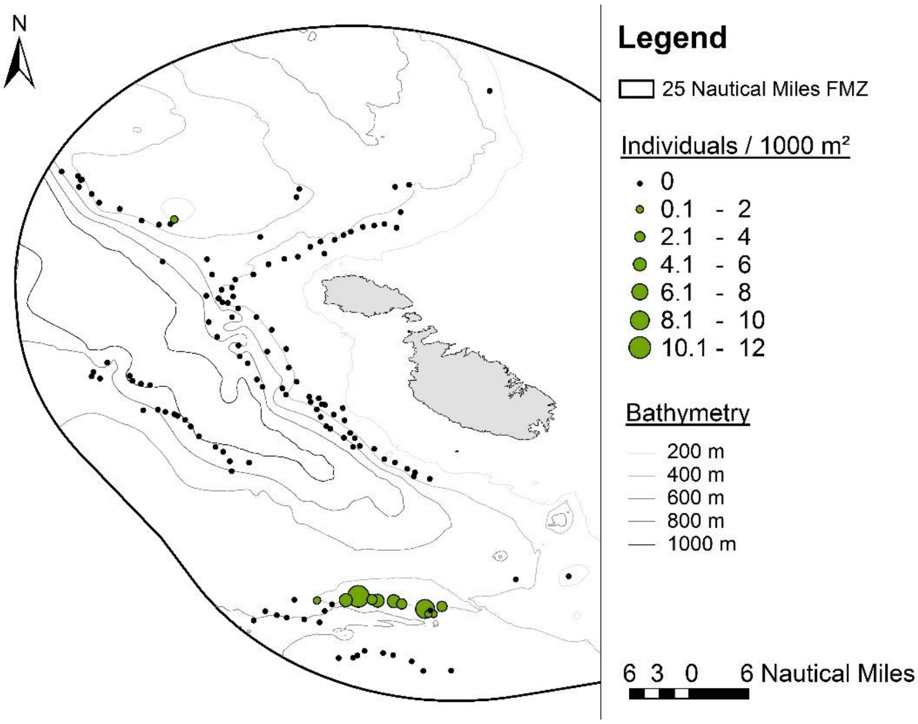


Fig. 33: Map of Malta showing the spatial distribution and density of *Coronaster briareus* individuals recorded through ROV dives within the 25 nautical miles FMZ.

Sclerasterias neglecta and *Sclerasterias richardi*

Bathymetric distribution

S. neglecta was recorded from 287m to 796m depth, and *S. richardi* from 245m to 469m depth*.* *S. richardi* may probably also be found at depths shallower than 200m because it was still frequent between 200m and 250m (Fig. 34). From these results, *S. neglecta* seems to have a wider depth range than *S. richardi*.

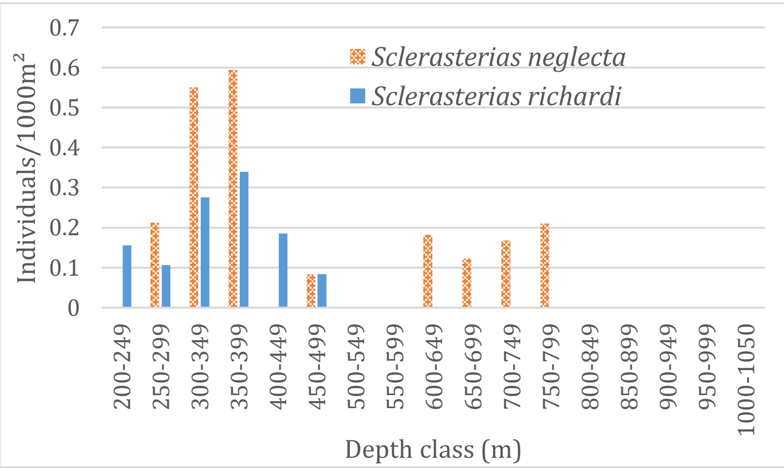


Fig. 34: Bathymetric distribution of *Sclerasterias neglecta* and *S. richardi*

Microhabitat

S. richardi was observed exclusively on rocky surfaces, while only 40% of *S. neglecta* individuals were on a rocky surface, the other 60% being found on the sediment.

Habitat type

S. neglecta seems to be associated more with sedimentary bottoms, while *S. richardi* was only found on rocky substrata, in areas with a mosaic of sediment and rocks, or rocks dominated by black coral (Fig. 35).

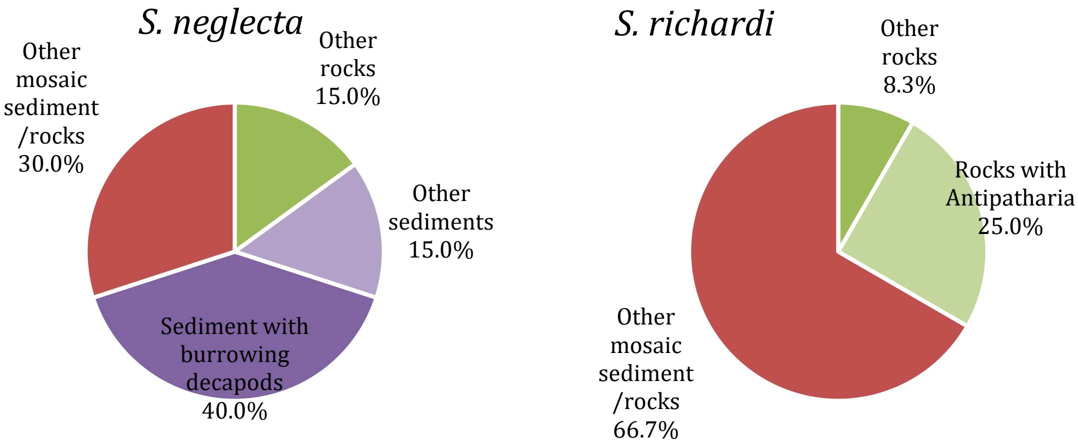


Fig. 35: Preferred habitat type of *Sclerasterias neglecta* and *S. richardi*

Spatial distribution

S. neglecta was present in 8.6% of the dives and *S. richardi* in 6.5% of all dives. Although not very frequent, they were distributed across the whole studied area and not restricted to one location (Fig. 36). Even if both species are closely related, they were not found in any of the same dives.

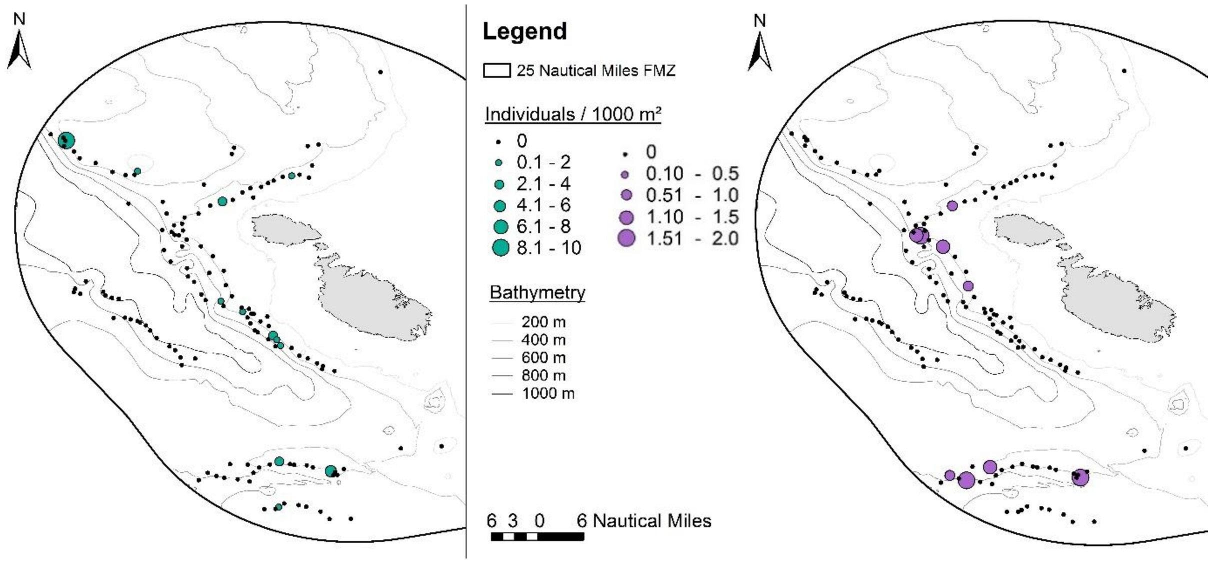


Fig. 36: Maps of Malta showing the spatial distribution and density of *Sclerasterias neglecta* (left) and *S. richardi* (right) individuals recorded through ROV dives within the 25 nautical miles FMZ.

Hymenodiscus coronata

Bathymetric distribution

Hymenodiscus coronata was found over a relatively wide depth range, between 304m and 666m depth, however not continuously (Fig. 37).

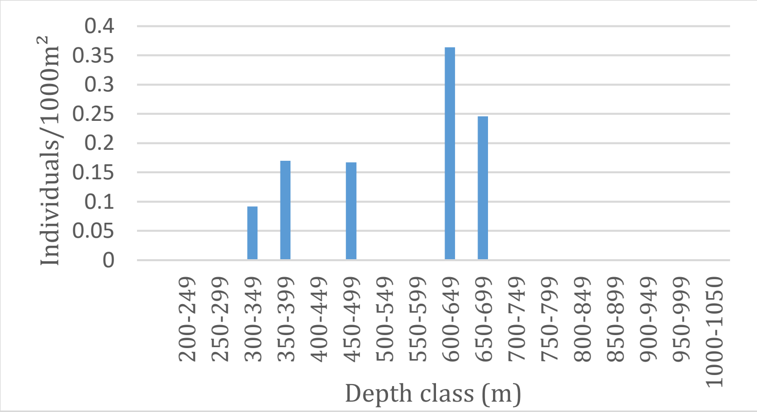


Fig. 37: Bathymetric distribution of *Hymenodiscus coronata*

Microhabitat and habitat type

Eight specimens were observed on sediment dominated by decapods, only one on rocks in a mosaic of sediments and rocks. Thus, *H. coronata* has a clear preference for loose sediments.

Spatial distribution

H. coronata was rare, always found as single individuals and was distributed in the northern and the southern part of the FMZ (Fig. 38).

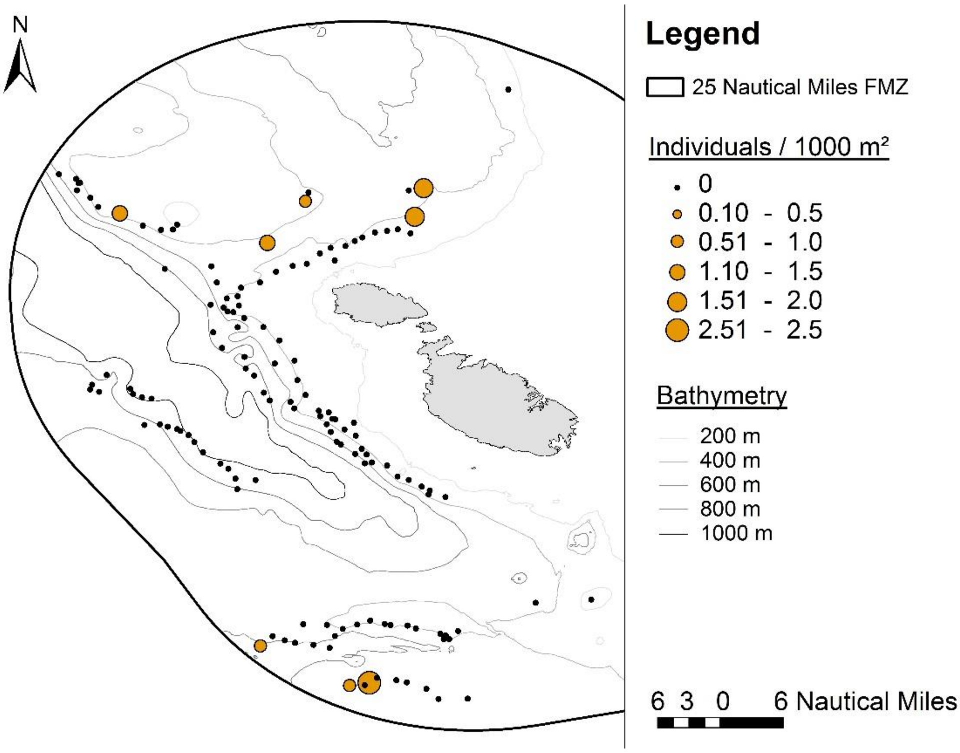


Fig. 38: Map of Malta showing the spatial distribution and density of *Hymenodiscus coronata* individuals recorded through ROV dives within the 25 nautical miles FMZ.

2.4. Holothuroidea

Mesothuria intestinalis

Bathymetric distribution

Most of the *M. intestinalis* individuals (72%) were found between 650m and 800m depth, but the full depth range was much larger: 331m to 919m (Fig. 39).

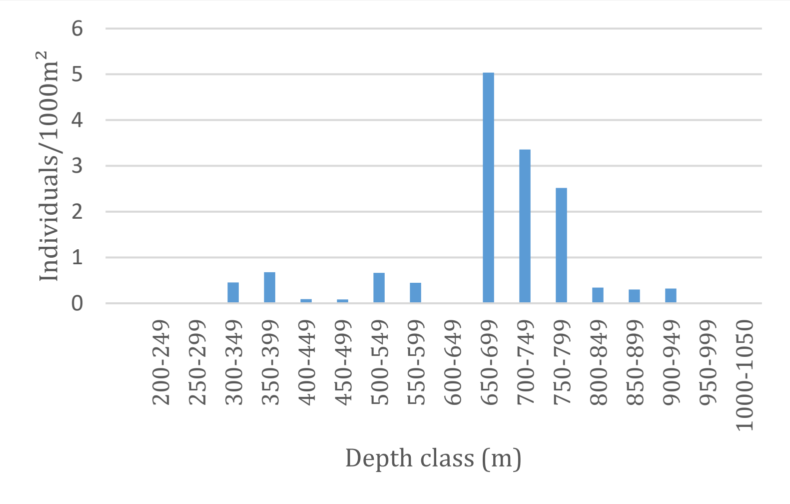


Fig. 39: Bathymetric distribution of *Mesothuria intestinalis*

Microhabitat and habitat type

M. intestinalis occurred exclusively on sediment as a microhabitat. It showed a clear preference for sedimentary habitats, mostly those dominated by burrowing decapods (Fig. 40). Only 4% of individuals were found in areas containing some rocks. In 5.9% of cases, *M. intestinalis* was the dominant species in the habitat.

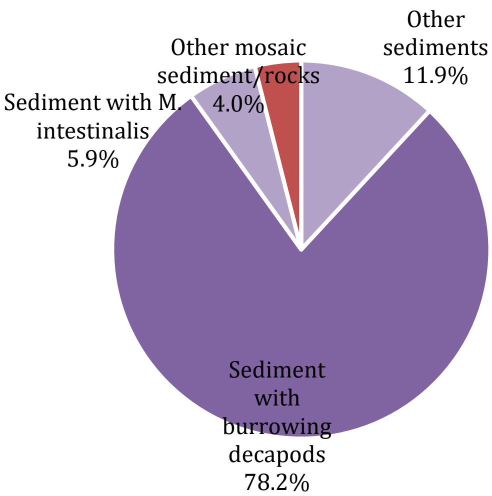


Fig. 40: Preferred habitat types of *Mesothuria intestinalis*

Spatial distribution

M. intestinalis was a relatively common species around Malta, occurring in 13% of all dives and distributed over the whole studied area (Fig. 41). In some areas, it formed large groups and was the dominant species. The largest density in a dive was 33 individuals/1000m^2.

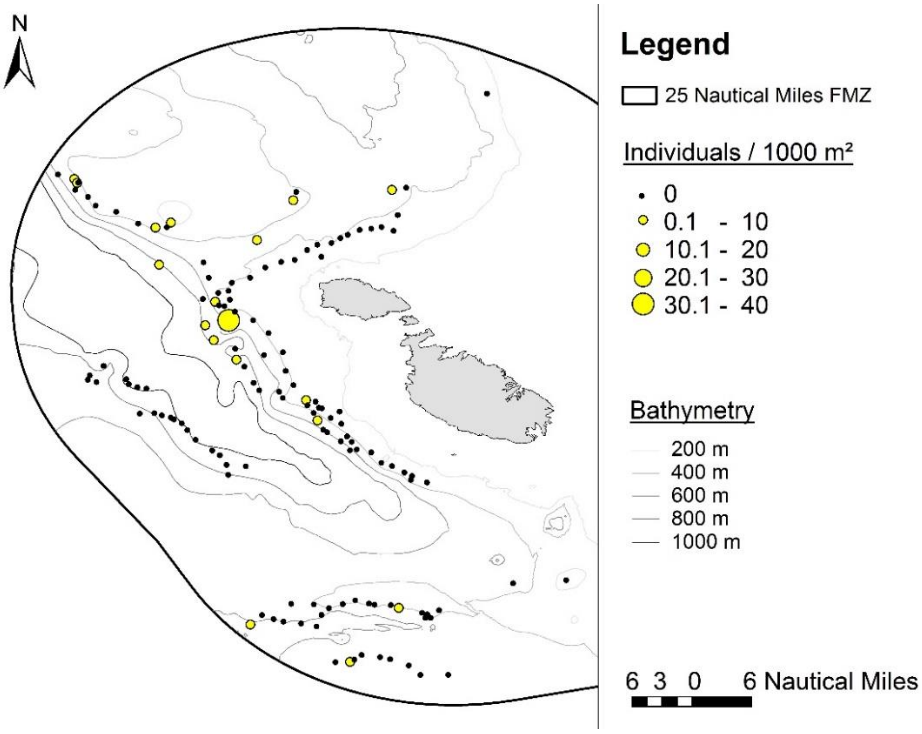


Fig. 41: Map of Malta showing the spatial distribution and density of *Mesothuria intestinalis* individuals recorded through ROV dives within the 25 nautical miles FMZ.

Other Holothuroidea

Five individuals of a greenish brown *Holothuria* sp. were found between 239m and 250m, in mosaic habitats of rocks and sediment, and showing no clear preference for a soft or hard substratum as their microhabitat.

Three individuals of *Parastichopus regalis* were found at depths of 246m to 274m, both on a rocky and sedimentary surface. A fourth individual was seen at 895m depth, but due to its different appearance and depth, it might have been a different species.

2.5. Ophiuroidea

***Ophiothrix* sp.**

Two different kinds of ophiuroids were identified as *Ophiothrix* sp., but had different morphologies; they were labelled 'red morph' and 'grey morph' (see Appendix 2 for pictures of each type).

Bathymetric distribution

As seen in Fig. 42, both 'types' of *Ophiothrix* seem to have a similar depth range, being more abundant in the shallowest depth class, and then decreasing in abundance with increasing depth. The deepest they were found was 413m for the red morph, and 411m for the grey morph. However, the red morph was more abundant in the deeper zones (250-350m) than the grey morph (Fig. 42).

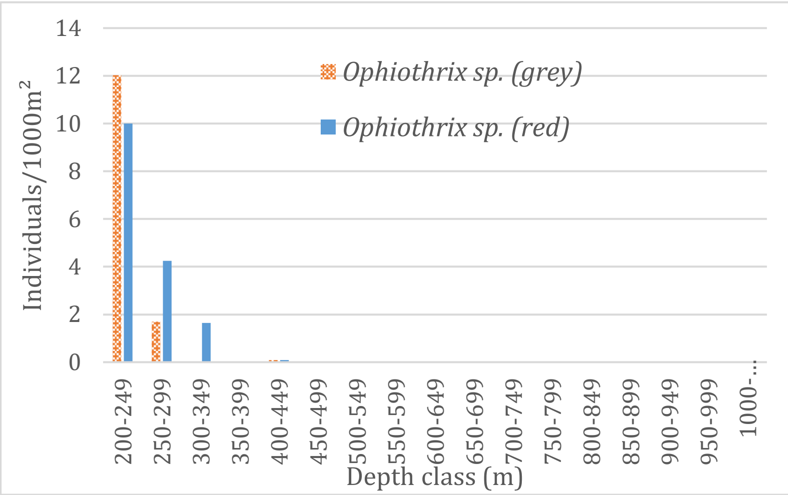
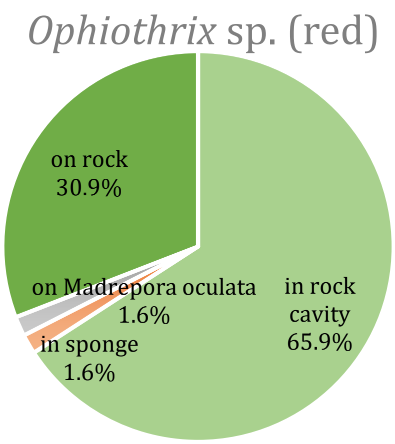


Fig. 42: Bathymetric distribution of the two colour morphs of *Ophiothrix* sp.

Microhabitat

The two 'types' of *Ophiothrix* sp. show a difference in preferred substrata; the red morph was mostly found in rock cavities with only its arms showing, and the grey morph was usually associated with the soft coral *Bebryce mollis* and sometimes other corals (Fig. 43). 38 individuals of the grey morph were found on 3 colonies of *Callogorgia verticillata*, but these might also be a different 'type' as they were white and not banded like the ophiuroids on *B. mollis*.



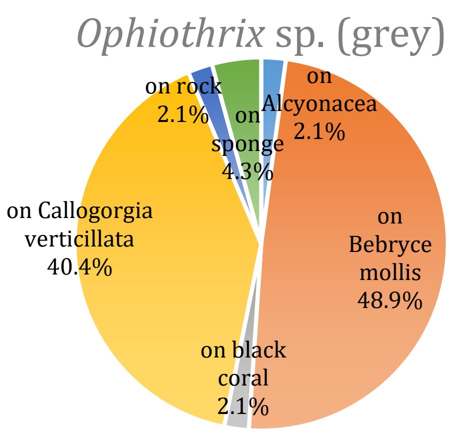


Fig. 43: Microhabitat of red and grey *Ophiothrix* sp.

Habitat type

The two 'types' of *Ophiothrix* also showed differences in habitat. As Fig. 44 shows, both seem to prefer mosaics of sediment and rocks and both were very rare on sedimentary bottoms, but the grey morph was associated with soft corals, while the red morph was mostly found in habitats dominated by crinoids, black corals or Alcyonacea and Demospongiae.

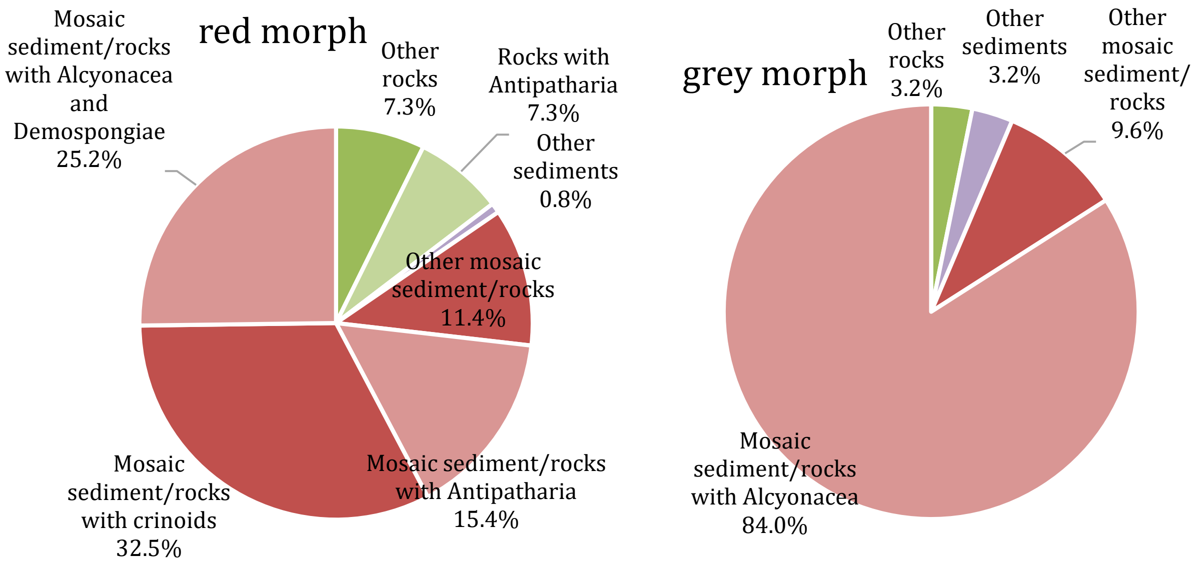


Fig. 44: Preferred habitat types of red and grey morphs of *Ophiothrix* sp.

Spatial distribution

Both 'types' seemed to be relatively common in Maltese waters (Fig. 45). The grey morph seemed to be more abundant to the North-west of the FMZ, whereas the red morph was more frequent south of Malta.

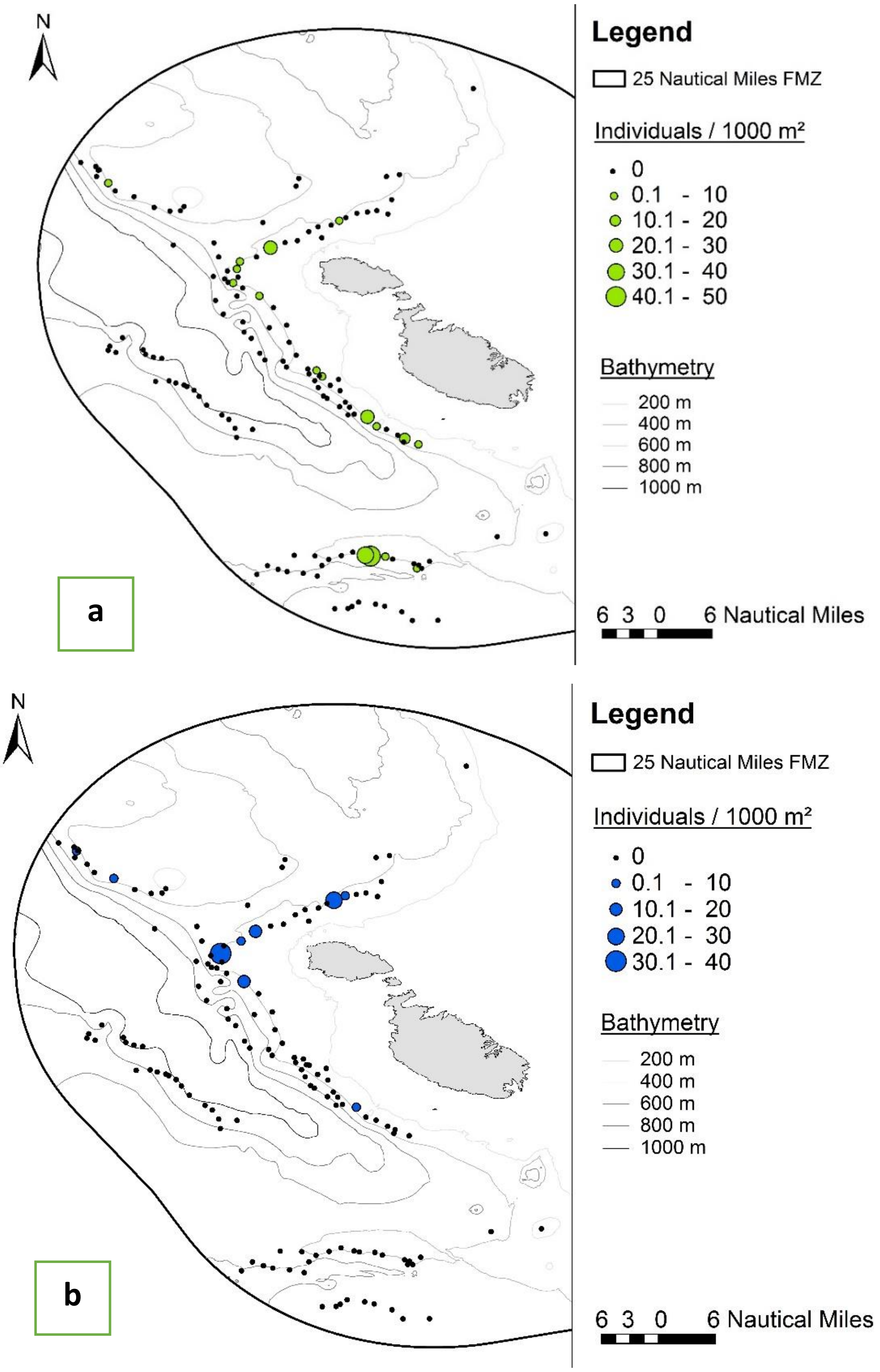


Fig. 45: Maps of Malta showing the spatial distribution and density of red morph (a) and grey morph (b) of *Ophiothrix* sp. individuals recorded through ROV dives within the 25 nautical miles FMZ.

Other ophiuroids

Three specimens of *Astrospartus mediterraneus* were found between 234m and 241m depth; two were attached to a dead black coral, the other to a specimen of *Bebryce mollis*, always in a mosaic of sediments and rocks dominated by corals. Two specimens of *Ophiura ophiura* were seen on sedimentary bottoms at 237m and 259m depth.

IV. Discussion

1. Limitations of this study

ROV surveys have many advantages, however, they collect data with a megafaunal and epifaunal bias [18]. This means that in the present study, species smaller than 4cm as well as juveniles were probably not seen, and infauna also remained unnoticed. In addition, the ROV does not zoom in on every individual, which results in low resolution images for the smaller species and individuals. Another problem is that when the ROV is in continuous movement, it is not possible to take a clear image of a specimen using the screen-grab function of the image analysis software. For example, in six cases, it could not be decided if a sea star was an *Odontaster mediterraneus* or a *Peltaster placenta* because the image resolution was too low to see the marginal plates. These individuals were excluded from the final analyses. In order to have a complete view of all the echinoderm fauna, one should combine ROV surveys with samples obtained by trawling and/or grabs, that will contain burrowing species, and perhaps more close-ups by the ROV. Furthermore, the ROV surveys of 2015 and 2016 took place in the months of June and July, which did not allow any potential seasonal changes in echinoderm populations to be studied.

Because the granulometry was not measured in this study, bottom types were only identified through ROV images as rocky substrata, fine sediment or coarse sediment. However, what appears as soft substratum might be quite coarse if inclusions such as shell fragments are present, which cannot always be seen in the ROV footage. Thus, there is a possibility that the bottom type was not always correctly recorded.

Human error should also be taken into account: even if the ROV videos were analyzed carefully, it is possible that some echinoderms have gone unnoticed thus decreasing the total abundance of specimens in the results.

Additionally, identification based on images alone can be quite problematic, because many determining criteria in echinoderms require microscopic examination. Some examples are the sclerites of holothuroids, and the pedicellariae of cidarids. For this reason, four kinds of echinoderms were only identified to genus. The images in appendix 2 are meant as a reference in case a more experienced taxonomist notices a misidentification.

2. Echinoidea

The sea urchins *Stylocidaris affinis* and *Cidaris cidaris* were the most widely distributed echinoderms, occurring in most of the dives across the FMZ. This was expected because both cidarids are collected frequently from the deep Mediterranean Sea [16]. *S. affinis* was also the most common echinoderm species sampled in Maltese waters during the trawling survey of the MARCOS cruise in 2007 [20]. Cidarids were found in every depth class surveyed at variable densities, and *C. cidaris* showed no increase or decrease in abundance with depth, thus no clear preference for shallower or deeper waters. Based on the present results, it cannot be confirmed that *S. affinis* has a smaller depth range than *C. cidaris*, as has been suggested in a previous study [34]. It can only be inferred that *S. affinis* is more frequent in shallower waters, which agrees with previous studies [34]. However, the distribution of *S. affinis* in this study does not stop at 1000m as is reported in Coll et al. [11]. *S. affinis* is still relatively abundant in the deepest depth stratum surveyed in this study (1000-1050m), which suggests that it could also be present deeper than 1050m. This is conceivable because in the Caribbean Sea, it was found down to depths of 1249m [36]. Previously, the MEDITS trawl survey observed *S. affinis* in Maltese waters from 50m to 550m, but predominantly between 50m and 200m, and *C. cidaris* from 50m to 700m [34]. The previous study also suggests that the distribution of the two cidarids is closely related to the bottom type: while both species co-occurred on coarse sediment at shallower depths, *C. cidaris* was found on fine sediment when it was the only cidarid, and *S. affinis* only occurred in deeper stations when the sediment had coarse inclusions [34]. The results in the present study were different; here both cidarids co-occurred on coarse and on fine sediment across all depth ranges, and *C. cidaris* was more frequently associated to coarse sediment. This difference might be due to a misidentification because the proportional spine length was the only criterion used to differentiate the cidarids. Unfortunately, the spines of juvenile *S. affinis* are proportionally longer than in adults, up to twice the diameter of the test [4], so those juveniles would probably have been identified as *C. cidaris* in the present study.

C. cidaris and *S. affinis* seem to be generalist species: they were found on various kinds of substratum and in every habitat type, without showing any strong preference. This agrees with literature reporting both cidarids on various substrata, particularly on muddy bottoms [31], on gravel, stone and coral [32]. Because *C. cidaris* is known to feed on sponges and gorgonians [31], one could expect it to occur mostly in habitats dominated by these groups, but this was not the case in the present study, presumably because it can feed on various other organisms. Both species of cidarids are known to carry epibionts such as zoanthids, serpulids, bryozoans, barnacles and small bivalves on their spines, they are thus important contributors of the overall biodiversity of their habitat [22][31].

Gracilechinus sp. proved to be a more common species in deeper water on rocky habitats, but especially those populated by cold-water corals like *Madrepora oculata*, and 40% of individuals were settled on colonies of coral species. This behaviour might be linked to their feeding habit, but despite the commonness of this genus, almost nothing is known of their biology and ecology [37].

Centrostephanus longispinus, which was very common in Maltese waters in Muscat's study, with a total abundance of 47,000 individuals/km^2[22], was only found once in the present study. However, in the previous study it was found between 72m and 171m depth, a depth range not surveyed here. Its reported depth range is down to 363m [11], although the specimen found in the present study occurred slightly deeper than this, at 379m. It appears that *C. longispinus* might become rarer in waters deeper than 200m.

3. Crinoidea

Crinoids differed from most of the other echinoderms recorded in that they can occur in very dense aggregations of thousands of individuals per 1000m^2 (up to 2937 individuals/1000m^2 in a dive). However, they were not widely distributed compared to other echinoderms and mostly occupied a small area to the south of the FMZ. This area could be qualified as a hotspot for crinoids in the FMZ. A previous Maltese study also found dense *A. mediterranea* and *L. phalangium* beds to the south of the FMZ, although not precisely in the same area as in the present study, but on sedimentary bottoms since the sampling was made by trawls [22]. These sediments contained shells and stones, presumably allowing crinoids to attach to the substratum [22]. The crinoids in the present study seemed to be specialised for rocky habitats where they can attach, and where they were usually the dominant species. Both species clearly have a different depth range, with *Antedon mediterranea* mostly occurring shallower than 300m, and *Leptometra phalangium* mostly between 400m and 600m. This depth segregation may prevent interspecific competition for the same resources.

Hebbeln et al. also found typical mass-occurrences of crinoids through ROV exploration in the Western Mediterranean; the species were resting on various substrata: soft sediment with coral rubble, the gorgonian *Callogorgia verticillata*, rocks, sponges (where crinoids attached to small irregularities), *Acanthogorgia hirsuta* and other coral species [38].

Crinoid beds of *Leptometra phalangium* are potential indicators of highly productive areas and are essential habitats for major commercial fish species [10]. Because of their suspension feeding, *L. phalangium* are indicators of bottom currents [10]. Crinoid beds also enhance the habitat heterogeneity to the same level as deep-water corals and gorgonids [10]. Unfortunately, they are likely to be destroyed by trawl fishing due to their fragile nature [10]. Protecting areas with *L. phalangium* beds would eventually lead to a higher survival rate of juveniles and spawners of commercial fish species and thus be beneficial for fishery management in the Mediterranean [10]. Therefore, the South of the FMZ, an area especially rich in crinoid beds, should be given particular attention in the future in terms of habitat conservation.

4. Asteroidea

Asteroids seem to be solitary animals, only very rarely occurring in the same frame as other sea stars. They occupied different habitats; *Peltaster placenta*, *Odontaster mediterraneus*, *Marginaster capreensis* and *Sclerasterias richardi* seem to be specialised for rocky habitats, whereas *Anseropoda placenta* and *Hymenodiscus coronata* are specialised for sedimentary bottoms. This agrees with literature: *H. coronata* and *A. placenta* are known to inhabit muddy and sandy bottoms [31]. *Sclerasterias neglecta* and *Coronaster briareus* seem to be more generalist, being found both on hard and on soft substrata. The most common sea star was *Peltaster placenta,* occurring in a third of all dives, in every depth class surveyed. In comparison, other sea stars such as *A. placenta*, *S. richardi* and *C. briareus* had a narrower depth range, with a span of less than 300m. Their habitat might be more restricted through environmental conditions.

While most sea stars occurred across the whole surveyed area, *Coronaster briareus* seems to be the only one showing a strong preference for one small geographic area to the South of the FMZ. Only one specimen was observed outside of this zone. The Maltese population found through the Life Baħar Project is to this date the only one recorded in the

Mediterranean [15]. In the Atlantic, *C. briareus* is more common in the western part than the eastern part, and confirmed records of this species closest to the Mediterranean were from the Great Meteor Seamount and Irving Seamount, located south of the Azores [15]. As this is almost 4000 km away from Malta, the origin of the Maltese population is still uncertain; it might have been introduced through human activities, arrived naturally through long distance larval dispersal or was always present in the deep Mediterranean, but never observed before [15]. Hebbeln et al. report a ROV sighting of *Coronaster* sp. in the Alboran Sea (Western Mediterranean), which has the same physical appearance as the individuals found in the present study [38]. However, since the specimen was not collected, it could not be identified to species level and does not confirm the presence of *C. briareus* in another part of the Mediterranean.

Marginaster capreensis was found for the first time in Maltese waters in this study, but only 4 specimens were observed. In the Mediterranean, this species is known from the Western Basin and the Aegean Sea, down to depths of 2487m [11]. Logically, because the Western Mediterranean and the Aegean Sea are connected through the Central Mediterranean, any species found in both regions is likely to also be present in the central region unless it has divided into two isolated populations. *M. capreensis* has probably always been present in the Central Mediterranean, be it in lower abundance or at deeper depths, which could be why it has not been recorded previously.

Sclerasterias neglecta is another sea star previously unknown in Maltese waters and in the Central Mediterranean. It was observed in the Western Basin, the Adriatic Sea and the Aegean Sea, from 160m to 485m depth [11]. Thus, it is not surprising that it would also be present in the Central Mediterranean, and probably not previously recorded because of limited biodiversity assessments. Yet according to Mastrototaro et al., there were as of 2008 no recent records of *S. neglecta* in the Mediterranean [21]. In the present study, *S. neglecta* also shows a larger depth range than the one given in Coll et al. of 160m to 485m [11]: it was seen from 287m to 796m depth. Following this, its depth range can be updated, and further deep-sea research in the Mediterranean would probably find *S. neglecta* at even greater depths, because in the present study there did not appear to be a decline in density with depth.

Sclerasterias richardi is considered a rarely occurring species, mostly because of its depth distribution and its habitat type [21]. Twenty-five specimens were recorded in 2007 to the west of Gozo, on a maerl substratum, between 135m and 208m [21]. The present study provides more evidence on the presence of this species in Maltese waters: it was found to the west of Gozo but also to the south of the FMZ, which suggests that it is not as rare as previously thought. It was here only observed on rocky substrata, between 245m and 469m, but considering the previous study this species is not exclusively found on rocks.

In the present study, *Hacelia attenuata* was found at 266m depth, which is deeper than its current depth limit of 190m [11]. This species is known to be sciaphilic and to feed mostly on sponges [4], thus, it is not unlikely that it could also be found naturally deeper than 190m. Similarly, *Peltaster placenta* also seems to have a larger depth range than previously recorded; in the present study, it was found down to 1020m depth. It was quite abundant deeper than 678m, its current depth limit in the Mediterranean [18], with a maximum density in the last depth stratum surveyed, from 1000m to 1050m. Therefore, *P. placenta* will very likely be found deeper than the limits of this survey. Its strong preference for rocky substrata is a possible reason for it not having been recorded at these depths in previous deep-sea trawling surveys, which generally target soft bottoms.

5. Holothuroidea

Mesothuria intestinalis is known in every region of the Mediterranean, from 18m to 4255m depth [11]. With 101 specimens observed in 13% of the dives, *M. intestinalis* proved to be a relatively common species around Malta. Because it was abundant in this study and because it only occurred on soft bottoms, it is surprising that *M. intestinalis* has never been found before in Maltese waters during trawling surveys. Dense aggregations of *M. intestinalis* in the Western Mediterranean have previously coincided with increased temperature, turbidity and abundance of labile organic matter [9]. According to a previous study, the peak biomass of *M. intestinalis* could be regulated by factors such as river inputs, even in communities as deep as 1600m [9]. This however does not apply to the Maltese islands, where there are no terrestrial inputs.

The greenish holothurian identified as *Holothuria* sp. could not be determined to the species level in the absence of actual specimens. It seems to be a generalist species, being found on soft sediment as well as on rocks. The same is true for specimens of *Parastichopus regalis*. *P. regalis* is usually considered a very common species on deep muddy bottoms [31]. Compared to *M. intestinalis*, *Holothuria* sp. and *P. regalis* were very rare in the present study. Other holothuroids that could inhabit the deep Mediterranean are mostly burrowing species [22], which would not have been seen in ROV footage. Hebbeln et al. observed that Mediterranean deep-water holothurians, occurring both on soft and hard substrata, often carried ophiuroids on them, but this association was not observed in the present study [38]. In the previous study, holothurians were not identified [38], but from the images provided they appear to be the same three species found in the present study.

6. Ophiuroids

Three different genera, and probably four different species, of ophiuroids were recorded in the deep sea in the FMZ. The red and grey morphs of *Ophiothrix* were difficult to identify because the image resolution was low and features such as spines were hard to count. In addition to their different colour, both morphs found in the present study occupied separate habitats; the red morph was usually found in rock cavities whereas the grey morph was almost exclusively found on soft coral species such as *Bebryce mollis*. This is not the only occasion an ophiuroid displayed a link to deep-water corals; an example is *Ophiotreta valenciennesi rufescens*, which is almost always associated with *Lophelia* corals [20]. Additionally, the red *Ophiothrix* morph is more abundant than the grey morph at greater depths, and inhabits an area to the south of Malta where no grey morph was found. All these differences suggest that they should not be treated as the same species.

Ophiothrix is a widespread genus in European waters and usually regarded as comprising two species, *O. fragilis* and *O. quinquemaculata*, but their status and that of their named sub-species are still debated, because of the variable morphologies [39]. Some authors unite both into one species: *O. fragilis* [4]. Tortonese also gives the possibility that *O. quinquemaculata* is a deep-water variation of *O. fragilis* [31]. Nonetheless, a recent genetic study found two different lineages of *Ophiothrix*, which were unrelated to the classic *O. fragilis* and *O. quinquemaculata* delimited on morphological criteria, and the two lineages can have overlapping features [39].

Hebbeln et al. also found large aggregations of red *Ophiothrix* in between dead coral frameworks such as in the present study, and described them as being about 30 cm in diameter, with their central disc generally hidden and arms outstretched [38]. Based on the provided images, those had the same physical appearance as the red morphs in the present study, and were identified as *O. fragilis* [38]. Because some specimens were sampled in the previous study, they might have been identified through physical examination, but this was not specified in the report. However, identification keys affirm that the size of *O. fragilis* should not exceed a maximum of 20mm for the disc and 100mm for the arms [31], whereas *O. quinquemaculata* can reach 15mm for the disc and 150mm for the arms [3], which is closer to the 30cm diameter ophiuroids found by Hebbeln et al. [38]. Tortonese describes *O. fragilis* as occurring in every type of habitat, often on or inside sponges, often hiding in cracks or empty shells, feeding on small invertebrates and detritus, and sometimes dead fish [31]. *O. quinquemaculata* is found mostly on hard substrata with two or three arms outstretched to catch food particles in the water current [31]. According to this, the grey *Ophiothrix* morph of the present study might come closer to *O. fragilis* and the red morph to *O. quinquemaculata*. Ultimately, because of overlapping features, a revision of the genus *Ophiothrix* based on molecular data will probably be necessary in order to correctly identify the species present in the Mediterranean.

The basket star *Astrospartus mediterraneus* was always observed perched on corals, presumably because it needs to elevate itself in order to capture suspended particles from the water; it was also often found on gorgonians and sponges in previous studies [40]. The three specimens were found slightly deeper than their current recorded depth limit of 188m [11], thus their depth range should be updated.

7. Total echinoderm fauna

Overall, the deep sea in the Maltese FMZ contains many echinoderms but is not very diversified. Four species only made up 92.83% of all observed echinoderms. These were in order of decreasing abundance *Antedon mediterranea*, *Leptometra phalangium*, *Stylocidaris affinis* and *Cidaris cidaris.*

In total, this study recorded 25 echinoderm taxa, including 21 species and three at generic level as the species level could not be identified from the images. This represents only 21 echinoderms out of the 77 species known from Maltese waters; the three new records are *Marginaster capreensis*, *Sclerasterias neglecta* and *Mesothuria intestinalis*. These species were here observed for the first time in Maltese waters, which shows that knowledge on the total biodiversity in an area is rarely complete, it can regularly be updated by further research. Another ROV survey in different locations of the FMZ would very likely lead to the discovery of other new species for Malta. Other than the crinoids, of which both known species were found here, sea stars were well represented in the present study (11 species out of 22 Maltese species), and other classes were represented in similar proportions in this study, as about one fourth of the Maltese species (5/23 echinoids, 3/12 holothuroids, 4/16 ophiuroids). The fact that only a subsample of all Maltese species was found in this study is consistent with the affirmation that benthic diversity decreases with increasing depth in the Mediterranean [18].

The total number of species per 1000m^2 did not decrease with depth; on the contrary, it was stable between 200m and 950m, then seemed to increase in the last two depth strata surveyed, between 950m and 1050m. However, this result is most likely an artefact of the unequal sampling effort in different depths. In fact, the diversity might seem to increase in the last two depth strata because those were significantly less surveyed than the others (see Fig. 4). While the shallower depth classes were each filmed on average over 7694m^2, only 813m^2 were filmed between 950m and 1000m, and 358m^2 between 1000 and 1050. Thus, the small number of species recorded at these depths yielded a high diversity when standardised according to the area sampled. Without this artefact, it is likely that the echinoderm diversity would remain relatively constant throughout the surveyed depths. This artefact also influences the bathymetric distribution graphs of individual species; the density of individuals in the last two depth classes are likely overestimated.

The relative evenness of echinoderm diversity across the surveyed depths does not support the findings of Terribile et al. [18], namely that total megabenthic diversity decreased with depth around the Maltese Islands. Perhaps echinoderms show different trends than other groups. Another possible reason for the decreasing biodiversity is that the previous study was based on trawling surveys over sedimentary bottoms, which generally host a reduced biodiversity in comparison to rocky habitats dominated by deep-water corals. As mentioned in the introduction, there are many theories that could explain an increasing or stable diversity in the deep sea, such as the stable environmental conditions and the heterogeneous seafloor. However, it is difficult to generalize deep sea biodiversity patterns because they largely depend on the ocean and the taxon concerned [17].

When comparing present results with those of the trawling surveys around Malta analysed by Terribile et al. [18], many species are the same in both studies. However, deeper than 200m, Terribile et al. also found *Marthasterias glacialis* and *Tethyaster subinermis* in small quantities [18], which were not observed in the present study. To the South of the FMZ, Mifsud et al. [20] recorded among others *Ophiotreta valenciennesi rufescens*, *Amphipholis squamata*, *Ophiocomina nigra*, *Amphiura chiajei, Amphiura filiformis, Marthasterias glacialis* and tests of *Echinocyamus pusillus* between 436m and 620m depth, which were all absent from the present study. These results show that the deep sea around Malta is more diverse than just the 25 taxa found in this study. The previous studies had different sampling methods than the present study and might have surveyed different habitats. Ophiuroids, which tend to be very small and hide under rocks, are presumably more likely to be caught through grabs and trawls, such as in Mifsud et al.'s study, than seen in ROV videos.

In this study, six species were found deeper than their currently accepted depth range. With ROV studies enabling research in deeper and more diverse environments than trawling surveys, the depth ranges of many species are likely to be updated in the future. Many echinoderms found in this study would probably also be found even deeper. An example is *Stylocidaris affinis*; according this study, *S. affinis* is still relatively abundant in the deepest depth class surveyed, which suggests that it could be present deeper than 1050m. In the present study, sea urchins were the class with the widest depth range; cidarids and *Gracilechinus* sp. occurred over the whole studied area, whereas brittle stars had the narrowest depth range; no brittle star was seen deeper than 413m. This might be because brittle stars are the most inconspicuous class of echinoderms and many small species would not have been seen in the ROV footage. Additionally, the size of an organism can influence its bathymetric distribution, larger animals being able to disperse more easily across various depths [13].

The present study allowed to gain insight on the microhabitat of many deep-sea echinoderms, which can only be observed in situ and is not recorded in trawling surveys. Because previous deep-sea studies were based on trawling samples, most echinoderms species found in the present study are reported in the literature as occurring on sedimentary bottoms. However, the present study reveals that many occur more often on rocky substrata, corals, or anthropogenic objects than on sediments.

Anthropogenic impacts

Echinoderms proved to occupy very varied habitats. Discarded anthropogenic objects such as limestone slabs and ropes from the FAD fishery provided colonizable substrata for many organisms. In this study *Stylocidaris affinis*, *Peltaster placenta*, *Cidaris cidaris*, *Antedon mediterranea*, *Leptometra phalangium*, *Coronaster briareus* and *Gracilechinus* sp. were all found on some occasions on anthropogenic substrata. In particular, limestone slabs were common anthropogenic objects in the present study. These are regularly used around the Maltese islands to anchor fish aggregating devices for the capture of *Coryphaena hippurus,* and are then left on the seafloor [41]. They can have negative effects by crushing benthic fauna, but generally they increase the biodiversity of sedimentary bottoms by providing colonizable islands of hard substrata for species usually found in rocky areas [41]. A previous Maltese study has shown that marine litter, usually viewed as pollution, can act as an artificial reef by supporting a more abundant but less diverse fauna than the surrounding sediment, comprised of different species than the sediment, which consequently increases the local biodiversity [42]. Discarded fishing gear was also observed to have negative impacts on the echinoderm fauna; many cidarids were seen entangled in ropes, probably trapped because of their spines. Crinoids however used ropes as a substratum to attach to, especially in sandy or muddy areas with few or no rocks, or when rocks were already saturated with crinoids.

Another anthropogenic threat is trawl fishing, which can greatly disturb the structure of benthic assemblages [18]. For instance, crinoids and ophiuroids make up an important part of the structuring fauna, and these show a significant decrease where there is frequent trawling [18]. Nonetheless, no evident sign of trawling was observed in the present study.

V. Conclusion

Ultimately, the objectives of this study were successfully reached. Twenty-five echinoderm taxa were identified from the deep FMZ, including three species which were new records for Maltese waters. This results in an updated checklist of 80 echinoderm species occurring in Maltese waters. Six species were observed deeper than before in the Mediterranean, which implies that their depth range can be updated through the findings of this study. In addition, extensive new data were collected on the abundance of these species at different depths, on their preferred microhabitats and bottom types. Echinoderms occurred in very varied habitat types due to their diverse ecology. Especially, deep-water coral reefs and rocky habitats dominated by scleractinians, black corals or gorgonians proved to host a large variety of echinoderm species. Spatial distribution maps were created for the 15 most common deep-water echinoderms, and show that the escarpments of the Malta Graben and the southern part of the FMZ are particularly rich in echinoderms. The southern part of the FMZ is especially important for hosting the large crinoid aggregations found in this study, as well as the only known population of *Coronaster briareus* in the Mediterranean.

In general, ROV surveys prove to be very useful; they deliver a significant amount of information concerning the fauna and the structure of the deep seafloor for less effort than other sampling methods. They can be used for biodiversity assessments of all megabenthic groups at once or separately and have recently led to the rediscovery of many marine taxa. They also provide ecological and behavioural information which can only be observed in situ and can allow us to improve our understanding of the deep sea.

The findings of the present study can improve our knowledge on deep-sea echinoderms in the Central Mediterranean, as well as helping the characterization of deep-sea ecosystems and habitats. Finally, they can be a source of information to help identify key areas for future conservation through designation of protected areas.

VI. References

[1] D. Pawson, "Phylum ECHINODERMATA," *Zootaxa*, vol. 1668, no. 3, pp. 749–764, 2007.

[2] S. Uthicke, B. Schaffelke, and M. Byrne, "A boom-bust phylum? Ecological and evolutionary consequences of density variations in echinoderms," *Ecol. Monogr.*, vol. 79, no. 1, pp. 3–24, 2009.

[3] R. Koehler, *Faune de France: Échinodermes*. Lechevalier, 1921.

[4] C. M. Tanti, "A taxonomic study of echinoderms of the Maltese isands and surrounding waters," unpublished MSc dissertation, University of Malta, 2003.

[5] University of Michigan, "ADW: Echinodermata: INFORMATION." [Online]. Available: http://animaldiversity.org/site/accounts/information/Echinodermata.html.

[6] C. T. Amemiya, T. Miyake, and J. P. Rast, "Echinoderms.," *Curr. Biol.*, vol. 15, no. 23, pp. R944-6, 2005.

[7] A. Matarrese, "Echinodermata," *Biol. Mar. Mediterr.*, vol. 17, no. 1, pp. 619–624, 2010.

[8] K. Iken, B. Konar, L. Benedetti-Cecchi, J. J. Cruz-Motta, A. Knowlton, G. Pohle, A. Mead, P. Miloslavich, M. Wong, T. Trott, N. Mieszkowska, R. Riosmena-Rodriguez, L. Airoldi, E. Kimani, Y. Shirayama, S. Fraschetti, M. Ortiz-Touzet, and A. Silva, "Large-Scale Spatial Distribution Patterns of Echinoderms in Nearshore Rocky Habitats," *PLoS One*, vol. 5, no. 11, 2010.

[9] J. E. Cartes, F. Maynou, E. Fanelli, C. Romano, V. Mamouridis, and V. Papiol, "The distribution of megabenthic , invertebrate epifauna in the Balearic Basin (western Mediterranean) between 400 and 2300 m : Environmental gradients in fl uencing assemblages composition and biomass trends," *J. Sea Res.*, vol. 61, no. 4, pp. 244–257, 2009.

[10] F. Colloca, P. Carpentieri, E. Balestri, and G. D. Ardizzone, "A critical habitat for Mediterranean fish resources: Shelf-break areas with Leptometra phalangium (Echinodermata: Crinoidea)," *Mar. Biol.*, vol. 145, no. 6, pp. 1129–1142, 2004.

[11] M. Coll, C. Piroddi, J. Steenbeek, K. Kaschner, F. B. R. Lasram, J. Aguzzi, E. Ballesteros, C. N. Bianchi, J. Corbera, T. Dailianis, R. Danovaro, M. Estrada, C. Froglia, B. S. Galil, J. M. Gasol, R. Gertwage, J. Gil, F. Guilhaumon, K. Kesner-Reyes, M. S. Kitsos, A. Koukouras, N. Lampadariou, E. Laxamana, C. M. L. F. de la Cuadra, H. K. Lotze, D. Martin, D. Mouillot, D. Oro, S. Raicevich, J. Rius-Barile, J. I. Saiz-Salinas, C. S. Vicente, S. Somot, J. Templado, X. Turon, D. Vafidis, R. Villanueva, and E. Voultsiadou, "The biodiversity of the Mediterranean Sea: Estimates, patterns, and threats," *PLoS One*, vol. 5, no. 8, 2010.

[12] S. C. Anderson, J. M. Flemming, R. Watson, and H. K. Lotze, "Serial exploitation of global sea cucumber fisheries," *Fish Fish.*, vol. 12, no. 3, pp. 317–339, Sep. 2011.

[13] R. Danovaro, J. B. Company, C. Corinaldesi, G. D'Onghia, B. Galil, C. Gambi, A. J. Gooday, N. Lampadariou, G. M. Luna, C. Morigi, K. Olu, P. Polymenakou, E. Ramirez-Llodra, A. Sabbatini, F. Sardá, M. Sibuet, and A. Tselepides, "Deep-sea biodiversity in the Mediterranean Sea: The known, the unknown, and the unknowable," *PLoS One*, vol. 5, no. 8, pp. 1–25, 2010.

[14] A. Koukouras, A. I. Sinis, D. Bobori, S. Kazantzidis, and M. Kitsos, "The echinoderm (Deuterostomia) fauna of the Aegean Sea , and comparison with those of the neighbouring seas," *J. Biol. Res.*, vol. 7, no. January, pp. 67–92, 2007.

[15] J. Evans, L. Knittweis, R. Aguilar, H. Alvarez, J. A. Borg, S. Garcia, and P. J. Schembri, "On the occurrence of Coronaster briareus (Echinodermata, Forcipulatida, Asteriidae) in the Mediterranean Sea," *Marine Biodiversity*, Marine Biodiversity, pp. 1–10, 2016.

[16] A. Mecho, D. S. M. Billett, E. Ramirez-Llodra, J. Aguzzi, P. A. Tyler, and J. B. Company, "First records, rediscovery and compilation of deep-sea echinoderms in the middle and lower continental slope of the Mediterranean Sea," *Sci. Mar.*, vol. 78, no. 2, p. 281, 2014.

[17] C. R. Mcclain and T. A. Schlacher, "On some hypotheses of diversity of animal life at great depths on the sea floor," *Mar. Ecol.*, vol. 36, no. 4, pp. 849–872, 2015.

[18] K. Terribile, J. Evans, L. Knittweis, and P. J. Schembri, "Maximising MEDITS: Using data collected from trawl surveys to characterise the benthic and demersal assemblages of the circalittoral and deeper waters around the Maltese Islands (Central Mediterranean)," *Reg. Stud. Mar. Sci.*, vol. 3, pp. 163–175, 2015.

[19] C. M. Tanti and P. J. Schembri, "A synthesis of the echinoderm fauna of the Maltese islands," *J. mar. biol. Ass. U.K.*, vol. 86, pp. 163–165, 2006.

[20] C. Mifsud, M. Taviani, and S. Stöhr, "Remarks on Echinodermata from the South Central Mediterranean Sea based upon collections made during the MARCOS cruise (10 to 20th April, 2007)," *Mediterr. Mar. Sci.*, vol. 10, no. 2, pp. 63–72, 2009.

[21] F. Mastrototaro and C. Mifsud, "Some observations on the morphology of Sclerasterias richardi a rarely encountered Mediterranean Sea star (Echinodermata: Asteriidae)," *Mediterr. Mar. Sci.*, vol. 9, no. 2, pp. 103–110, 2008.

[22] K. Muscat, "Spatial and bathymetric distribution of deep-water megabenthic echinoderms in GSA 15," *Unpubl. BSc Diss. Univ. Malta*, 2016.

[23] M. Taviani, L. Angeletti, A. Ceregato, B. Gualandi, E. Lipparini, and D. Malatesta, "The crinoid garden of Montecristo Island Marine Sanctuary (Tuscan archipelago National Park , Mediterranean Sea)," *Rapp. Comm. int. Mer Médit.*, vol. 39, p. 674, 2010.

[24] M. Bo, S. Canese, C. Spaggiari, A. Pusceddu, M. Bertolino, M. Angiolillo, M. Giusti, M. F. Loreto, E. Salvati, S. Greco, and G. Bavestrello, "Deep Coral Oases in the South Tyrrhenian Sea," *PLoS One*, vol. 7, no. 11, 2012.

[25] "Malta Fisheries Mgt Zone - Biology - University of Malta." [Online]. Available: https://www.um.edu.mt/science/biology/staff/profpatrickschembri/empafish/malta_fisheries_mgt_zone. [Accessed: 13-Mar-2017].

[26] "Fisheries Management Plan - Scientific Technical and Economic Committee for Fisheries - Joint Research Centre - European Comission." [Online]. Available: https://stecf.jrc.ec.europa.eu/documents/43805/595618/Maltas+Fisheries+Management+Plan+-+Trawler+and+Lamapra.pdf. [Accessed: 13-Mar-2017].

[27] M. Camilleri, M. Dimech, and A. Drago, "Spatial distribution of demersal fishery resources, environmental factors and fishing activities in GSA 15 (Malta Island)," *MedSudMed Tech. Doc.*, vol. 15, no. 13, p. 97, 2008.

[28] "About | Natura 2000 Networking Programme." [Online]. Available: http://www.natura.org/about.html. [Accessed: 18-Jun-2017].

[29] "Natura 2000 - Environment - European Commission." [Online]. Available: http://ec.europa.eu/environment/nature/natura2000/index_en.htm. [Accessed: 18-Jun-2017].

[30] "Life Bahar for Natura 2000 - Biology - University of Malta." [Online]. Available: http://www.um.edu.mt/science/biology/staff/life_bahar_for_natura_2000. [Accessed: 10-Mar-2017].

[31] E. Tortonese, *ECHINODERMATA, Fauna d'Italia 6*. Bologna: Calderini, 1965.

[32] E. C. Southward, A. C. Campbell, L. S. of London, and E. and C. S. Association, *Echinoderms: Keys and Notes for the Identification of British Species*, no. 56. Linnean Society of London and the Estuarine and Coastal Sciences Association, 2006.

[33] T. Mortensen, *Handbook of the echinoderms of the British Isles*. Oxford university Press, 1927.

[34] K. Terribile and P. J. Schembri, "Depth distribution of Cidaris cidaris (Linnaeus, 1758) and Stylocidaris affinis (Philippi, 1845)(Echinodermata, Echinoidea) around the Maltese Islands.," *Rapp. Comm. int. Mer Médit.*, vol. 40, p. 697, 2013.

[35] CoCoNet project, "Revised list of Mediterranean and Black Sea habitats," 2016. [Online]. Available: http://cordis.europa.eu/result/rcn/181925_en.html.

[36] Smithsonian National Museum of Natural History, “Invertebrate Zoology Collections Search.” [Online]. Available: http://collections.nmnh.si.edu/search/iz/?q=qn+Stylocidaris+affinis#new-search. [Accessed: 23-Jun-2017].

[37] J. M. González-Irusta, I. Preciado, L. López-López, A. Punzón, J. E. Cartes, and A. Serrano, “Trawling disturbance on the isotopic signature of a structure-building species, the sea urchin Gracilechinus acutus (Lamarck, 1816),” *Deep. Res. Part II Top. Stud. Oceanogr.*, vol. 106, pp. 216–224, 2014.

[38] D. Hebbeln, C. Widenberg, L. Beuck, A. Freiwald, and P. Wintersteller, “Report and preliminary results of RV POSEIDON Cruise POS 385 “Cold-Water Corals of the Alboran Sea (western Mediterranean Sea),” *Berichte aus dem Fachbereich der Geowissenschaften der Univ. Bremen*, no. 273, pp. 1–79, 2009.

[39] R. Pérez-Portela, V. Almada, and X. Turon, “Cryptic speciation and genetic structure of widely distributed brittle stars (Ophiuroidea) in Europe,” *Zool. Scr.*, vol. 42, no. 2, pp. 151–169, 2013.

[40] H. Zibrowius, “Observations biologiques au large du Lavandou (côte méditerranéenne de France) à l’aide du sous-marin Griffon de la marine nationale,” *Trav. sci. Parc nation. Port.cros*, vol. 4, pp. 171–176, 1978.

[41] R. Pace, M. Dimech, M. Camilleri, and A. Cabalenas Mosteiro, “Distribution and density of discarded limestone slabs used in the traditional Maltese lampuki fishery,” *Rapp. Comm. int. Mer Médit.*, vol. 38, p. 568, 2007.

[42] R. Pace, M. Dimech, M. Camilleri, and P. J. Schembri, “Litter as a source of habitat islands on deep water muddy bottoms,” *Rapp. Comm. int. Mer Médit.*, vol. 38, no. June, p. 567, 2007.

Appendix

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Appendix 1: Geographical positions of ROV dives

2015 Dives	Latitude	Longitude	2015 Dives	Latitude	Longitude
1	35.89282	14.11716	**1.5**	35.93449	14.05914
3	35.44495	14.24290	**1.6**	35.92748	14.12807
5	36.18765	14.31536	**1.7**	35.85601	13.88426
6	36.16767	13.92941	**1.8**	35.90509	13.86605
7	36.14628	14.08002	**1.9**	35.97883	14.00793
11	35.77405	14.28777	**11.1**	35.79133	14.27126
12	35.50379	14.06903	**11.2**	35.79475	14.23555
1.1	35.86991	14.16462	**11.3**	35.79618	14.24672
1.10	35.96423	14.04367	**11.4**	35.80984	14.22036
1.11	35.87588	14.17816	**11.5**	35.80878	14.23862
1.12	35.88230	14.12309	**12.1**	35.51468	14.37014
1.13	35.83887	14.22108	**12.2**	35.52750	14.38456
1.14	35.87851	14.16225	**12.3**	35.54408	14.24413
1.15	35.90744	14.07457	**5.1**	36.22999	14.30582
1.16	35.90896	13.81185	**5.2**	36.23336	14.32940
1.17	35.82918	14.19068	**5.3**	36.16304	14.25175
1.18	35.85955	14.21826	**6.1**	36.16701	13.91046
1.19	35.99059	14.09955	**6.2**	36.17535	13.93613
1.2	35.90359	14.14118	**7.1**	36.10950	14.12065
1.20	36.01191	14.07378	**7.2**	36.22650	14.14576
1.21	35.94601	14.04566	**7.3**	36.10010	14.09411
1.22	35.89488	14.08397	**7.4**	36.08318	14.06849
1.23	35.84485	14.18120	**7.5**	36.03586	14.02576
1.24	35.86594	14.18366	**7.6**	36.21260	14.14082
1.25	35.82465	14.19744	**7.7**	36.11289	14.14298
1.3	35.79445	14.00518	**7.8**	36.07465	14.03834
1.4	35.75355	14.03207	**9**	36.39069	14.46460

2016 Dives	Latitude	Longitude	2016 Dives	Latitude	Longitude
A01	35.95394	14.09204	**B11**	35.89792	13.89531
A02	35.95872	14.12349	**B12**	35.92001	13.80070
A03	36.01185	14.03271	**B14**	35.91256	13.79747
A04	36.02664	14.04300	**B15**	35.91362	13.86172
A05	35.76850	14.30581	**B16**	35.90025	13.88013
A06	35.75166	14.33934	**B17**	35.93568	13.82382
A07	35.74087	14.36411	**C01**	36.12944	14.16382
A08	36.19337	13.84479	**C02**	36.11797	14.18773
A09	36.20365	13.81042	**C03**	36.15477	14.23185
A10	36.24203	13.78129	**C04**	36.14132	14.20463
A11	36.24157	13.77935	**C05**	36.13834	14.18105
A12	35.81787	14.23070	**C06**	36.16786	14.28827
A13	35.75786	14.32645	**C07**	36.16154	14.30846
A14	35.74531	14.33739	**C08**	36.16513	14.27121
A15	35.84864	14.20319	**C09**	36.14927	14.21967
A16	35.85722	14.17498	**D01**	35.57753	14.59681
A17	35.86504	14.18860	**D02**	35.57253	14.50799
A18	36.03678	14.01644	**E01**	35.45288	14.25507
A19	36.06127	14.03276	**E02**	35.44546	14.30252
A20	36.05760	14.01525	**E03**	35.51957	14.18791
A21	36.04290	14.01024	**E04**	35.50550	14.15373
A22	36.04638	14.03472	**E05**	35.53835	14.13726
A23	36.00356	13.99382	**E06**	35.52305	14.35601
A24	36.08302	13.99953	**E07**	35.52042	14.36469
A25	36.04747	13.98952	**E08**	35.51475	14.36162
A26	36.10880	13.99081	**E09**	35.53134	14.31699
A27	36.10467	13.91656	**E10**	35.53604	14.30368
A28	36.17373	13.88168	**E11**	35.53793	14.22335
A29	36.21817	13.79767	**E12**	35.53691	14.27666
A30	36.22994	13.77623	**E13**	35.51364	14.10737
A31	36.25566	13.74735	**E14**	35.51936	14.08838
A32	36.24819	13.77503	**E15**	35.50857	14.12442
B01	35.85303	13.92124	**E16**	35.53084	14.20037
B02	35.84937	13.93624	**E17**	35.44896	14.28595
B03	35.85679	13.90883	**E18**	35.44068	14.21127
B04	35.76822	14.06133	**E19**	35.43511	14.33371
B05	35.78632	14.01801	**E20**	35.41967	14.39961
B06	35.77055	14.02933	**E21**	35.41945	14.35312
B07	35.81240	13.97762	**E22**	35.44126	14.23570
B08	35.84639	13.94144	**E23**	35.50045	14.17946
B09	35.82865	13.96364	**E24**	35.53852	14.26727
B10	35.83988	13.95434	**E25**	35.53727	14.17504

Appendix 2: Additional photos of echinoderms found in present study, for reference

Asteroidea

Fig. 1: Stillshot from the ROV footage of sea star identified as *Anseropoda placenta.*

Fig. 2: Stillshot from the ROV footage of sea star identified as *Astropecten ?irregularis*.

Fig. 3: Stillshot from the ROV footage of sea star identified as *Coronaster briareus.*

Fig. 4: Stillshot from the ROV footage of sea star identified as *Hacelia attenuata*.

Fig. 5: Stillshot from the ROV footage of sea star identified as *Hymenodiscus coronata*.

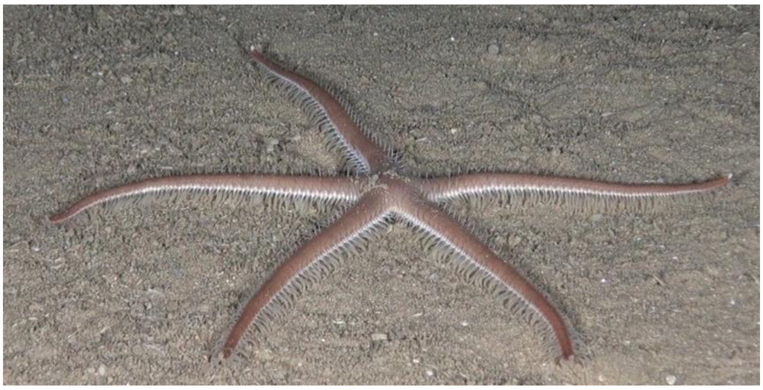

Fig. 6: Stillshot from the ROV footage of sea star identified as *Luidia sarsii*.

Fig. 7: Stillshot from the ROV footage of sea star identified as *Marginaster capreensis*.

Echinoidea

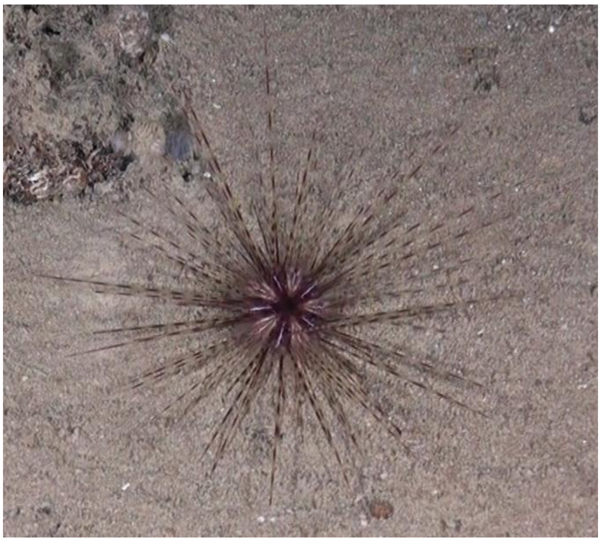

Fig. 8: Stillshot from the ROV footage of sea urchin identified as *Centrostephanus longispinus.*

Holothuroidea

Fig. 9: Stillshot from the ROV footage of holothurian identified as *Holothuria* sp.

Fig. 10: Stillshot from the ROV footage of holothurian identified as *Mesothuria intestinalis.*

Fig. 11: Stillshot from the ROV footage of holothurian identified as *Parastichopus regalis.*

Ophiuroidea

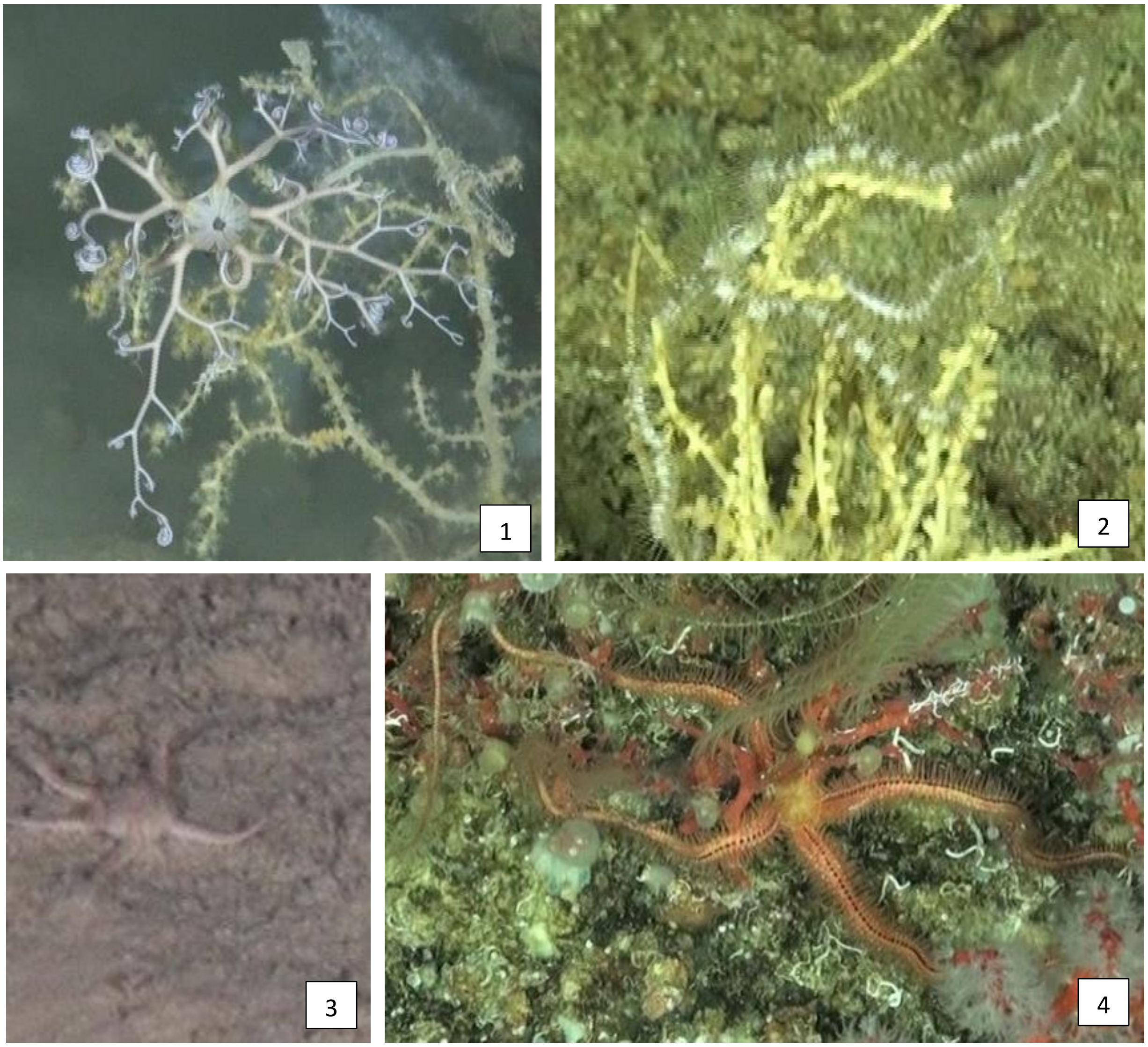

Fig. 12: Stillshots from the ROV videos of ophiuroids identified as 1. *Astrospartus mediterraneus,* 2. *Ophiothrix* sp. (grey morph), 3. *Ophiura ophiura,* 4. *Ophiothrix* sp. (red morph)

Appendix 3: List of habitat types used in present study

Bathyal rocks and other hard substrates

- Bathyal rocks
  - Bathyal rocks with Anthozoa
    - Bathyal rocks with Alcyonacea
      - Bathyal rocks with *Corallium rubrum*
      - Bathyal rock with *Muriceides lepida* and/or *Bebryce mollis* and/or *Villogorgia brevicoides*
      - Bathyal rock with *Viminella flagellum* and/or *Callogorgia verticillata*
      - Bathyal rocks with *Swiftia* sp.
    - Bathyal rocks with Antipatharia
      - Bathyal rocks with *Leiopathes glaberrima* and/or *Antipathes dichotoma*
    - Bathyal rocks with Scleractinia
      - Bathyal rocks with *Dendrophyllia cornigera*
      - Bathyal rocks with *Madrepora oculata* and/or *Lophelia pertusa*
    - Bathyal rocks with Scleractinia and Alcyonacea
      - Bathyal rocks with *Madrepora oculata* and/or *Lophelia pertusa* and *Corallium rubrum*
    - Bathyal rocks with Scleractinia and Tetractinellida
      - Bathyal rocks with *Madrepora oculata* and/or *Lophelia pertusa* and/or *Desmophyllum dianthus* with *Pachastrella monilifera* and/or *Poecillastra compressa*
  - Bathyal rocks with Demospongiae
    - Bathyal rocks with Tetractinellida
    - Bathyal rocks with desma-bearing demosponges (ex-”Lithistida”)
  - Bathyal rocks with Crustacea
    - Bathyal rocks with Balanomorpha
  - Bathyal rocks with Echinodermata
    - Bathyal rocks with Antedonoidea
      - Bathyal rocks with *Leptometra phalangium* and/or *Antedon mediterranea*
  - Bathyal rocks with Alcyonacea and Demospongiae
- Bathyal bioconstructions
  - Bathyal Scleractinia “reefs”
    - Bathyal *Madrepora oculata* and/or *Lophelia pertusa* and/or *Desmphyllum dianthus* “reefs”
- Anthropic substrates (wrecks, lost fishing gears, marine litter, submerged infrastructures)

Bathyal coarse sediment

- Bathyal coarse sediment with Hydrozoa

Bathyal sediments

- Bathyal sediments with Anthozoa
  - Bathyal sediments with Alcyonacea
    - Bathyal sediments with *Isidella elongata*
    - Bathyal sediments with *Bebryce mollis*
    - Bathyal sediments with *Swiftia* spp.
  - Bathyal sediments with Pennatulacea

    - Bathyal sediments with Scleractinia
        - Bathyal sediments with *Dendrophyllia cornigera*
    - Bathyal sediments with Actinaria
        - Bathyal sediments with *Actinauge richardi*
    - Bathyal sediments with Ceriantharia
        - Bathyal sediments with *Arachnanthus* and/or *Cerianthus* and/or *Pachycerianthus*
- Bathyal sediments with Hydrozoa
- Bathyal sediments with Crustacea
    - Bathyal sediments with Decapoda
        - Bathyal sediments with *Polycheles typhlops* and/or *Nephros norvegicus* and/or *Aristeus antennatus* and/or *Aristeomorpha foliacea* and/or *Aristeus antennatus* and/or *Bathypterois mediterraneaus* and/or *Nezumia sclerorhynchus* and/or *Nettastoma melanurum* and/or *Etompterus spinax*
- Bathyal sediments with Desmospongiae and Foraminifera
- Bathyal sediments with Echinodermata
    - Bathyal sediments with Mesothuriidae and/or Elasipoda
        - Bathyal sediments with *Mesothuria intestinalis*
- Bathyal sediments with Brachiopoda
    - Bathyal sediments with Terebratulidae
        - Bathyal sediments with *Gryphus vitreus*

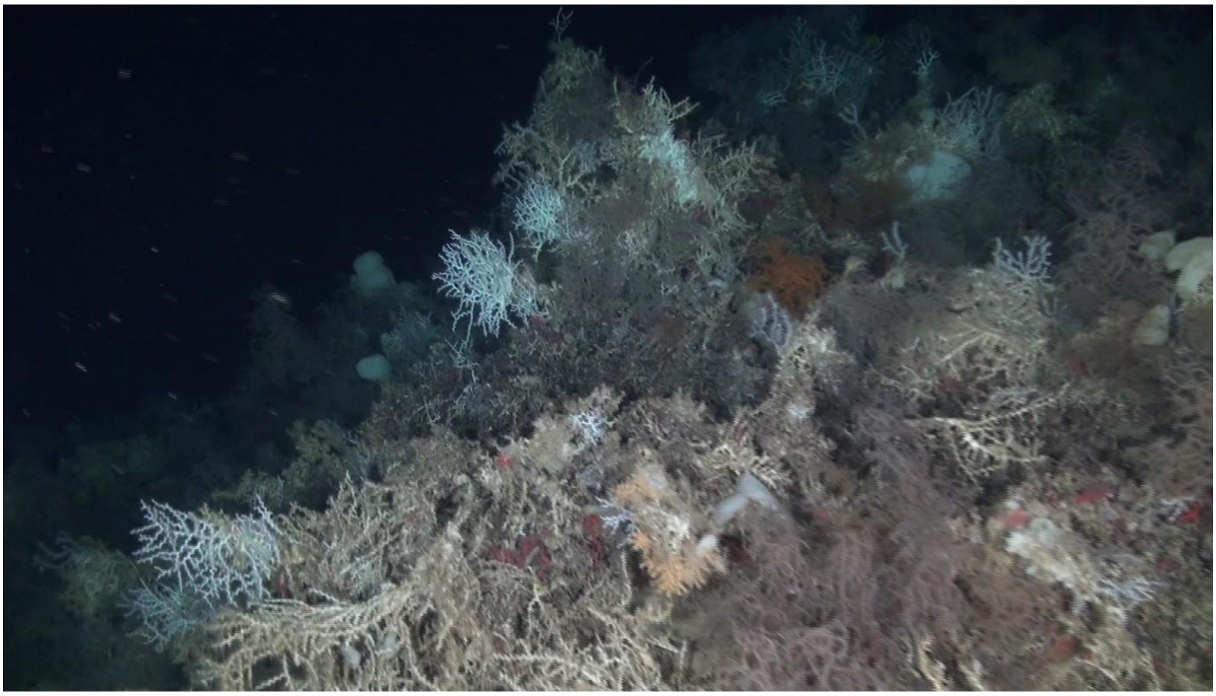

Fig. 13: Example of Bathyal *Madrepora oculata* and/or *Lophelia pertusa* and/or *Desmphyllum dianthus* “reefs”

Fig. 14: Example of Bathyal rocks with *Madrepora oculata* and/or *Lophelia pertusa* and/or *Desmophyllum dianthus* with *Pachastrella monilifera* and/or *Poecillastra compressa*

Fig. 15: Example of bathyal rocks with Alcyonacea and Demospongiae

Fig. 16: Example of bathyal sediment with Desmospongiae and Foraminifera

Fig. 17: Example of bathyal sediment with Hydrozoa

Appendix 4: Updated list of echinoderms present in Maltese waters

[18][15][19][20][21] ([e] stands for endemic to the Mediterranean)

Class ASTEROIDEA

Anseropoda placenta (Pennant, 1777)
Asterina gibbosa (Pennant, 1777)
Asterina pancerii (Gasco, 1870) [e]
Astropecten aranciacus (Linnaeus, 1758)
Astropecten bispinosus (Otto, 1823) [e]
Astropecten irregularis pentacanthus (Delle Chiaje, 1825) [e]
Astropecten jonstoni (Delle Chiaje, 1825) [e]
Astropecten platyacanthus (Philippi, 1837) [e]
Astropecten spinulosus (Philippi, 1837) [e]
Chaetaster longipes (Retzius, 1805)
Coronaster briareus (Verrill, 1882)
Coscinasterias tenuispina (Lamarck, 1816)
Echinaster sepositus (Retzius, 1783)
Hacelia attenuata Gray, 1840
Hymenodiscus coronata (G. O. Sars, 1872)
Luidia ciliaris (Philippi, 1837)
Luidia sarsi Lutken, 1858
***Marginaster capreensis* (Gasco, 1876)**
Marthasterias glacialis (Linneaus, 1758)
Odontaster mediterraneus (von Marenzeller, 1893)
Ophidiaster ophidianus (Lamarck, 1816)
Peltaster placenta (Müller & Trochel, 1842)
***Sclerasterias neglecta* (Perrier, 1891)**
Sclerasterias richardi (Perrier, 1882)
Tethyaster subinermis (Philippi, 1837)

Class CRINOIDEA

Antedon mediterranea (Lamarck, 1816) [e]
Leptometra phalangium (J. Müller, 1841) [e]

Class ECHINOIDEA

Arbacia lixula (Linnaeus, 1758)
Arbaciella elegans Mortensen, 1910
Brissopsis lyrifera (Forbes, 1841)
Brissus unicolor (Leske, 1778)
Centrostephanus longispinus (Philippi, 1845)
Cidaris cidaris (Linnaeus, 1758)
Echinocardium cordatum (Pennant, 1777)
Echinocardium flavescens (O. F. Müller, 1776)
Echinocardium mediterraneum (Forbes, 1844)
Echinocyamus pusillus (O. F. Müller, 1776)
Echinus melo (Lamarck, 1816)
Eucidaris tribuloides (Lamarck)
Genocidaris maculata A. Agassiz, 1869
Gracilechinus acutus Lamarck, 1816
Neolampas rostellata A. Agassiz, 1869
Paracentrotus lividus (Lamarck, 1816)
Plagiobrissus costai (Gasco, 1876)
Prionocidaris baculosa (Lamarck, 1816)
Psammechinus microtuberculatus (Blainville, 1825)
Schizaster canaliferus (Lamarck, 1816)
Spatangus purpureus (O. F. Müller, 1776)
Sphaerechinus granularis (Lamarck, 1816)
Stylocidaris affinis (Philippi, 1845)

Class HOLOTHUROIDEA
Holothuria forskali Delle Chiaje, 1823
Holothuria helleri Marenzeller, 1878 [e]
Holothuria impatiens (Forsskål, 1775) [e]
Holothuria mammata Grube, 1840 [e]
Holothuria polii Delle Chiaje, 1823
Holothuria sanctori Delle Chiaje, 1823
Holothuria tubulosa Gmelin, 1788
Leptosynapta minuta (Becher, 1906)
***Mesothuria intestinalis* (Ascanius, 1805)**
Stichopus regalis (Cuvier, 1817)
Trachythyone elongata (Düben-Koren, 1844)
Trachythyone tergestina (M. Sars, 1857) [e]
Trochodota venusta (Semon, 1887) [e]

Class OPHIUROIDEA
Amphipholis squamata (Delle Chiaje, 1828)
Amphiura brachiata (Montagu, 1804)
Amphiura chiajei Forbes, 1843
Amphiura filiformis (O. F. Müller, 1776)
Astrospartus mediterraneus (Risso, 1826)
Ophiacantha setosa (Retzius, 1805)
Ophiocomina nigra (Abildgaard in O. F. Müller, 1789)
Ophioderma longicaudum (Retzius, 1805)
Ophiomyxa pentagona (Lamarck, 1816)
Ophiopsila annulosa (M. Sars, 1857)
Ophiopsila aranea Forbes, 1843
Ophiothrix fragilis (Abildgaard, 1789)
Ophiothrix quinquemaculata (Delle Chiaje, 1828)
Ophiotreta valenciennesi rufescens (Koehler, 1896)
Ophiura albida Forbes, 1839
Ophiura grubei Heller, 1863
Ophiura ophiura (Linnaeus, 1758)