Effects of seasonal disturbance on the benthic assemblages of Maltese sublittoral cobble beds.

BACHELOR'S THESIS

Submitted on

Anhalt University of Applied Science Department of Agriculture, Ecotrophology and Landscape Development Supervisor: Prof. Dr. Erik Arndt

Submitted by

Gregor Boerner

Matr.nr.4050828

July 2013

Conducted at Department of Biology, University of Malta Supervisor: Prof. Dr. Patrick J Schembri

Eidesstattliche Erklärung

Declaration: I declare that this document and the accompanying code has been composed by myself, and describes my own work, unless otherwise acknowledged in the text. It has not been accepted in any previous application for a degree. All verbatim extracts have been distinguished by quotation marks, and all sources of information have been specifically acknowledged. I hereby certify that this dissertation has been composed by me and is based on my own work, unless stated otherwise. This work has not been submitted for any other degree.

Ich versichere, dass ich die vorliegende Arbeit selbstständig verfasst, in gleicher oder ähnlicher Fassung noch nicht in einem anderen Studiengang als Prüfungsleistung vorgelegt und keine anderen als die angegebenen Hilfsmittel und Quellen (einschließlich der angegeben oder beschriebenen Software) benutzt habe.

Berlin, 26.97.2013

Gregor Boerner

Acknowledgement

Foremost, I would like to express my sincere gratitude to my supervisor in Malta, Prof. Patrick J. Schembri, who offered me the interesting bachelor's topic for the continuous support of my study and research.

My sincere thanks also goes to my German supervisor Prof. Erik Arndt, who made the contact and this experience possible for me.

Furthermore I would like to thank Julian Evans for his patience, motivation, enthusiasm, and immense knowledge. Without his encouragement and guidance this thesis would not have been possible. I could not have imagined having a better advisor and mentor for my study.

I thank my fellow labmates in Malta, Juan Jose Bonello, Leanne Bonnici, Hazel Farrugia and Kimberly Terribile for all the support, help in the lab, for the stimulating discussions and as well for the good time in Malta.

I also would like to thank Nick Scott and Rachael Dickie for their linguistic corrections and Madleen Wernicke for the help with the design.

Last but not least, I would like to thank my family for all the support.

i. Abstract

The mollusc and crustacean assemblages of cobble bed habitats in the Mediterranean area are understudied. In this work, a description of seasonal changes in community composition of sublittoral cobble bed habitats in Malta (central Mediterranean) is presented for the first time. This study examines the effects of storms and the associated disturbance on biota living in this environment. Cobble and sediment samples were collected and sorted at the end of September (before the winter season when storms are frequent) and early April (after the storm season), in order to look for seasonal effects. Some species settle into these habitats during the spring and summer months, but fail to persist over the winter (for example, *Leptochelia savignyi*); others seem to be adapted to live in these highly dynamic environments (for example *Melita hergensis*) and were present throughout the year. Community structure differed between the two seasons; some species, such as *Gammarella fucicola*, were highly abundant in spring but populations decreased drastically during the winter, while others, for example *Ischnochiton rossoi*, disappeared completely. In contrast to that, species such as *Gibbula varia* increased slightly in abundance following the winter. The data presented corroborate the hypothesis that only highly adapted animals can survive this harsh environment throughout the whole year.

i Abstract

Table of Contents

Chapter 1: Introduction

1.1. Introduction	
1.2. Aims and Objectives of this study	4
Chapter 2: Materials and Methods	
2.1. Sampling sites selection	5
2.2. Field sampling procedure	
2.3. Laboratory analysis	
2.3.1. Sampling treatment	12
2.3.2. Sorting	
2.3.3. Identification	13
2.4. Grain size analysis	
2.5. Data analysis	
2.6. Multivariate analysis	
2.6.1. Similarity Profiles (SIMPROF)	
2.6.2. Similarity Percentages (SIMPER)	
2.6.3. Non-Metric Multidimensional Scaling (nMDS)	
Chapter 3: Results	
3.1. Cobble bed profile	
3.2. Physical Characteristics	18
3.3. Biological Characteristics	19
3.3.1. Species Richness	19
3.3.2. Total Abundance	21
3.4. Multivariate Analysis	23
Chapter 4: Discussion	
4.1. Context	29
4.2. Conclusion	32
ii References	

iii Appendix

1.1. Introduction

Sublittoral cobble beds are defined as a type of unconsolidated bottom, characterized by the dominance of cobble- and gravel-sized particles (gravel 2–63mm, cobbles 64–200 mm; ISO 14688-1), admixed with finer sediment and with a lack of large permanent surfaces for plant and animal attachment. Such beds appear in areas with lower energy than rocky bottoms but are still very unstable habitats, exposed to waves, currents, temperature changes and intense light penetration (Cowardin 1979). These habitats are formed by coastal processes that erode and deposit sediment along shorelines e.g. wind, waves, currents and tides. Depending on the relative contribution of the different processes, the sediment may form shingle beaches or remain in the sublittoral areas as submerged cobble habitats.

Around the Maltese Islands, both shingle beaches and shallow water cobble beds occur. While shingle and sandy beach habitats in the Maltese Islands are well investigated (Deidun et al. 2007), very little is known about infralittoral cobble bed habitats. The same is true for the Mediterranean area as a whole, where cobble habitats have attracted little attention. On a world wide scale some studies have been performed, but mostly these focus on either macrophytes, or on single species, or on sessile species and/or the effects of disturbance. However, these published studies at least give an idea regarding the factors affecting the communities living in cobble habitats.

Peres (1967) described cobble bed communities as biocoenosis dominated by the two characteristic amphipods *Melita hergensis and Parhyale aquilina*, which feed on organic detritus together with the fish species *Gouania wildenowii*, as a predator on those amphipods. Moreover, *Xantho poressa* is a crab species characteristic of these habitats as are several turbellarians and nemertines. Peres also mentioned that with increasing pebble size the described biocoenosis is impoverished but becomes populated by species from adjacent rock habitats.

While searching for the early benthic phase of the European lobster, Linnane (2001) determined that Crustacea are the most numerically abundant group in cobble sites. Because of the natural profile of the substratum, it offers many interstitial spaces for different faunal species with different requirements for protection. He came to the conclusion that these habitats are very important as nursery areas for juvenile lobsters by providing shelter.

Robinson and Tully (2000) came to the same conclusion while surveying the seasonal variations in community structures and recruitment of benthic decapods in subtidal cobble habitats. They noted that some species settle in cobble habitats, but fail to survive the winter while other species were found throughout the whole year.

While surveying algal diversity in subtidal cobble habitats, Davies and Wilce (1987) noted that these habitats are a very unstable mosaic of successional stages where the cobble size in association with the disturbance regime is the key factor influencing change. They also found that cobbles provide refuge for transient species and suggested that in order to survive these harsh conditions, species adopt different strategies. Thus, these authors conclude that cobble habitats are mainly characterized by disturbance.

There are several forms of 'disturbance'; in general the term is used for any force that removes biomass, and therefore includes 'biological disturbance' such as the effects of grazing and predation, for example (Witman 1985). However, in the present study 'disturbance' is taken to mean principally the overturning of cobbles and boulders by waves generated by winter storms.

Working on the effects of disturbance on the species diversity of a boulder field, Sousa (1979) considered overturning as the most influentially form of disturbance in those habitats. The rate of overturning linked to disturbance is negatively-correlated to the cobble size (Osman 1977). Scheiberling (2009) also noted that disturbance is the key to understanding the interactive mechanisms regulating the development and maintenance of cobble macroalgal community structure.

Likewise, working off Ghana, Lieberman & John(1979) found a correlation between the number of species per cobble and the cobbles' resistance to tumbling. He also discovered that the tumbling rate was higher during the rainy season. Lieberman compared the colonisation of the cobbles by a variety of life forms before and after the rainy season and noted significant seasonal variations in species richness and total biomass. Both values decrease in the rainy season when the tumbling rate of the cobbles increases. However, he also determined that the species diversity in cobble habitats was higher than on rocky reefs.

In marine epifaunal communities inhabiting unstable rocky substrata, predation plays a minor role in affecting community structure, which is mainly determined as competition for sheltered space (Osman 1977). In such communities, colonization is highly variable and could change seasonally, such that the main factors influencing community structure are larval selectivity, biological interactions focusing on the competition for space, the substratum particle size, seasonality, and physical disturbance as a function of the frequency of overturn; all these variables together produce a system of great variety (Osman 1977).

In summary, the published work shows that cobble bed habitats are very unstable places to live in. The main factor affecting attached organisms and temporary residents is disturbance. How the disturbance affects the motile inhabitants has not yet been discovered. In addition, the community is influenced by biological interactions - mainly competition relating to protected space. These two key factors, disturbance and competition, together with the available shelter resulting from the architecture of the cobble beds, are the key factors determining community composition.

However, how the whole community deals with the extreme conditions in cobble bed habitats and the changes in community composition between the seasons has never been recorded. Lieberman & John (1979) already worked out that the tumbling rate of the cobbles is higher during the rainy season in West Africa, which is comparable to the winter season in Malta, and Osman (1977) assumed that the communities change seasonally. Peres (1967) suggested that in the Mediterranean, during the stormy season the biota of pebble communities moves to the lower parts of the boulders generally mixed with the pebbles, or to deeper bottoms, to escape the moving pebbles and to come back as soon as it gets calm again. However, there have been no contemporary studies on how enhanced tumbling does affects motile organisms, how they handle the rapid shifts in the availability of space, and how the community structure changes during the stormy winter seasons.

1.2. Aims and Objectives of this study

Considering the above mentioned points, the aim of this study was to investigate the effect of churning action from waves on the cobble bed biota. The hypothesis is that some of the biota that settled on the cobbles during the summer months would be wiped out by the mechanical movement of cobbles as a consequence of higher disturbance during winter storms, so that only those forms adapted to live in the highly dynamic environment of the cobble beds would survive from year to year.

This hypothesis was tested by:

- collecting standardized samples of cobble sediment from before and after the winter season
- sorting out the biota
- identification and counting
- data analysis

2.1. Sampling site selection

A number of shallow-water cobble bed habitats occur around the Maltese Islands. These are mostly located between rocky shores or small shingle beaches and deeper water soft bottoms. After due consideration of depth, ease of access and location around the Island, the following sampling sites were selected for study.

Ta' Fra Ben (Figs 1, 2)

The Ta' Fra Ben sampling site (Fra Ben) is a small cove within Salina Bay, which is situated in northern Malta, close to Bugibba. The cove is located at the entrance of Salina Bay and is shielded by a small peninsula to the north. Since it is shallow, well protected from the prevailing wind, and surrounded by many hotels, the cove is a popular bathing spot.

Salina Bay faces east which means that it is directly affected by the Maltese Gregale, a strong north-eastern wind that blows in the central and western Mediterranean area. This may have a significant effect on Ta' Fra Ben particularly during the winter months.

The cobbles are located at a depth of 1 m, at a distance of approximately 3 m from the shore. The seabed is characterized by an abundance of rocks, with a layer of boulders and cobbles over pebbles, sand and dead *Posidonia oceanica*. The bed is between 4 cm and 7 cm thick.

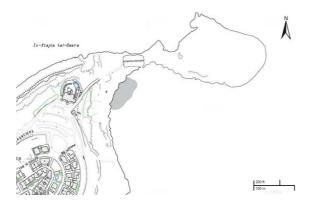




Fig. 1 Map of the Ta'Fra Ben area with the grey indicated sampling area (Map source: Mepa)

Fig.2 General view of the Ta' Fra Ben sampling site

II-Hofra z-Zghira (Fig. 3, 4)

II-Hofra z-Zghira (Hofra) is a small inlet in the southeast of Malta, situated between Marsaskala and Marsaxlokk. It is surrounded by low cliffs, which make it difficult to reach. Because of this, it remains relatively unaffected by people. However, it is close to the Delimara Power Station, and coolant water from the power station is discharged into it. This causes a year-round increase in the water temperature at this site.

The shore is dominated by cobbles, but also includes boulders, while *Posidonia oceanica* beds are present in deeper waters. The cobbles extend from the shore to the sublittoral reaching a depth of 0.5 m, at a distance of around 1 m from the coast. The average bed thickness is approximately 6 - 7 cm.

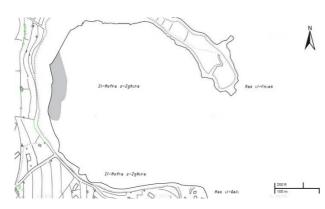


Fig.3 Map of II-Hofra z-Zghira area with grey indicated sampling area (Map source: Mepa)

Fig.4 General view of the II-Hofra z-Zghira sampling site

Ix-Xatt I-Ahmar (Fig.5, 6)

The Ix-Xatt I-Ahmar sampling site (Xatt I-Ahmar) is positioned on the southern coast of Malta's sister island, Gozo, facing Comino and Malta. This small cove has its mouth facing southwest and, despite the protection of a small peninsula to the south, Ix-Xatt I-Ahmar is directly affected by the Lbic – a hot east-southern wind bringing dust-laden, humid air.

Ix-Xatt I-Ahmar is a renowned diving spot because of its close proximity to wrecks and is also a popular bathing spot in summer.

The seabed is composed primarily of fine sandy sediment with an overlying layer of cobbles at a depth of 2 m that give rise to a sparse cobble bed having a thickness of 4 cm.

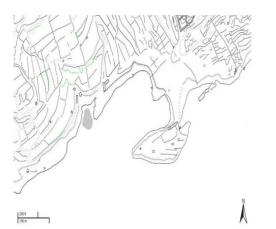


Fig.5 Map of Ix-Xatt I-Ahmar area with grey indicated sampling area (Map source; Mepa)



Fig.6 General view of the Ix-Xatt I-Ahmar sampling site

Ix-Xoqqa A. (Fig. 7, 8)

In the south of Malta, surrounded by the Malta Freeport and other industrial installations is the Ix-Xoqqa A. (Xoqqa A.) sampling site. Located in a small narrow creek facing south, Xoqqa A. is influenced by the Nofsinnhar wind, a normally warm wind blowing from the south, which also brings hot, humid, dusty air.

The site is very well protected on three sides and does not attract much boat traffic or human activity. A good amount of cobbles line the shore at a depth of approximately 50 cm with a bed thickness ranging from 4-7 cm.

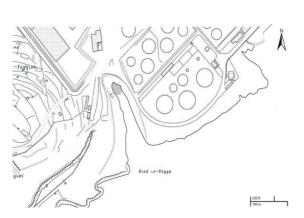


Fig.7 Map of Ix-Xoqqa A. area with grey indicated sampling area (Map source: Mepa)



Fig.8 General view of Ix-Xoqqa A. sampling site

Marsaxlokk (Fig.8, 10)

The Marsaxlokk sampling site is in the southeast of Malta, between the villages of Marsaxlokk and Birzebbuga. The cobble bed sampled was located between the old fishing harbour and the Malta Freeport container terminal, facing the Delimara Power Station.

The beach is partially sandy and situated between low cliffs on one side and a man-made harbour on the other. The seabed is dominated by boulders with small patches of cobbles found between the boulders, and which therefore do not form a continuous bed. The average thickness of the cobble patches is about 5 cm and they are found at a depth of about 1 m.

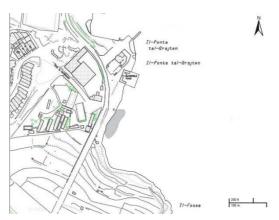


Fig.9 Map of the Marsaxlokk area with grey indicated (Map source: Mepa)



Fig.10 Marsaxlokk sampling site sampling area

It-Tunnara (Fig. 11, 12)

Located on the east side of Mellieha Bay in the north of Malta, close to Mellieha beach, It-Tunnara (Tunnara)_is a popular bathing spot. Facing northeast, Tunnara is also directly affected by the dry Grigal wind which blows with great force and causes high turbulence in the shallows. This sampling site lies adjacent to a small jetty in the east.

The seabed at Tunnara is mainly composed of cobbles and some large rocks, at a depth of 1 m; the cobble bed thickness is up to 10 cm.

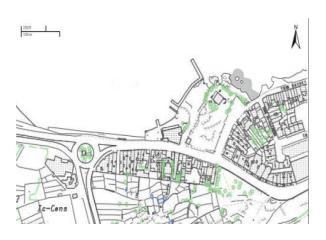


Fig.11 Map of the Tunnara area with grey indicated sampling area (Map source: Mepa)



Fig.12 General view of the Tunnara sampling site

2.2 Field sampling procedure

Sampling was carried out in October 2012 for the autumn samples and in March/April 2013 for the spring samples. At each sampling site, four randomly placed replicate samples were collected from within the cobble beds.

For each replicate, a circular corer of 35 cm diameter was placed on the area with cobbles. The cobbles were carefully removed by hand and transferred into a mesh bag of 1mm mesh made of inert material. A hand net was used to collect the finer sediment after removal of the cobbles and also placed in the mesh bag. Following collection, the mesh bags were placed in appropriate sample containers (10L) together with sea water. After collecting the samples, the depth of the cobble layer at each sampling spot was measured using a 30cm ruler.



Fig. 13 Sampling equipment



Fig. 14 Sample container including cobbles

2.3 Laboratory analysis

2.3.1 Sampling treatment

The samples were taken to the lab for initial processing on the same day. To prevent the samples from decomposing, they were transferred from the mesh bag to a container and covered with seawater-formalin (10% dilution) and stored in a cool dark place.

2.3.2 Sorting

To sort the samples they were first rinsed to get rid of the formalin solution. This was accomplished by washing the cobbles with water on a 0.5 mm sieve in a fume hood. The larger cobbles were transferred directly into a bucket of fresh water for rinsing and then placed in an empty container; whereas the sediment was transferred in portions to the sieve and puddled in a basin of water for several minutes before being placed in another container. To prevent decomposition these samples were stored in a cold room (approximately 14°).

After rinsing, the sample was sorted in small portions to make sure that all the biota was collected. The sediment was transferred onto a white tray and observed systematically to collect biota; these were separated into major taxa (Mollusca, Crustacea, Polychaeta and Echinodermata) and stored in 70% ethanol.



Fig. 15 Rinsing procedure



Fig. 16 Sorting procedure

2.3.3. Identification

Identification was made with the aid of a stereomicroscope. Before counting the molluscs the empty shells were removed. The shells were examined under the microscope using strong backlighting to verify if there were any soft body parts inside, and any empty shells were discarded. All the live molluscs were then individually examined, identified to species level and counted.

Crustaceans were also identified to species level and counted. On the other hand, the other taxa could not be included due to time constraints. The mollusc and crustacean data were recorded as number of individuals of each species per sample in a species-by-sample data matrix.



Fig.17 Amphipod crustacean under the microscope



Fig. 18 Xantho pilipes in preserved in Ethanol

2.4. Grain size analysis

Grain-size analysis was carried out by manually sorting the cobbles using a sizing template (Hydro Scientific Ltd.) with size fractions of 2-4, 4-8, 8-16, 16-32, 32-64 and 64-128mm. Each sample was sorted separately and the mass of each size class was then measured. The highest weight fraction represents the most abundant cobble size of each sample.

2.5. Data analysis

The faunal data were compiled for each sample site and subdivided by season of collection. Descriptive statistics including species richness and total abundance were calculated for each sample and graphs representing the variation of these parameters were constructed. To compare the abundances of species per site and season, Analysis of Variance (ANOVA) was used to test for differences in biological characteristics between the samples, sampling sites and seasons. Prior to the analyses, data were checked for normality and homogeneity of variances using Cochran's test and appropriate transformations were applied when the raw data did not meet this assumption.

All procedures mentioned were carried out using Microsoft Office Excel 2007version 6.1.7, except for ANOVA which was carried out using GMAV5 software (University of Sydney, Australia).

2.6. Multivariate analysis

Q-mode multivariate analysis was used to explore correlations in the assemblage structure between the sites and seasons. The analyses were made using PRIMER v6 (Plymouth Routines in Multivariate Ecological Research; Clarke &Gorley, 2006). A triangular matrix of similarities was computed using the Bray-Curtis similarity coefficient, which delivers robust and reliable results to express relationships in ecology (Clarke, Somerfield & Chapman, 2006).

To lessen the effect of very abundant single species, the data were transformed using a square root transformation before calculating the similarity coefficients. This transformation still places the emphasis on the most abundant species, but their importance is lessened due to the fact that even the medium-abundant species are considered (Clarke & Warwick, 2001).

Cluster analysis was carried out to find natural groupings and to divide them in a way so that the samples within a particular group are more similar to each other than to other groups. Cluster analysis was carried out using hierarchical group-average linkage, and a dendrogram was generated.

2.6.1. SIMPROF

Similarity Profiles (SIMPROF) were carried out to test for evidence of structure in an unstructured set of samples in order to determine if there are significant sub-group structures within cluster groups. First, a resemblance profile is determined by ranking the resemblance matrix of the data. A mean profile is then calculated by randomising the order of each variable's value and re-calculating the profile. The *pi*-statistic is calculated as the deviation of the actual data profile from the mean one. This is compared with the deviations of further randomly generated profiles to test for significance (Clarke et al., 2008). The SIMPROF analysis is a test for structure in the data, in order to deter over-splitting of clusters. On the other hand, it is still appropriate to define cluster groups at a higher similarity level if these are biologically meaningful (Clarke et al., 2008). In this case, a threshold at the similarity level of 30% was used to determine the cluster groups.

2.6.2. Similarity Percentages (SIMPER)

A SIMPER analysis was carried out to identify the species that are most important in creating the observed pattern of similarity, using the Bray-Curtis measure of similarity. This analysis breaks down the contribution of each species to the observed similarity (or dissimilarity) between the samples.

2.6.3. Non-Metric Multidimensional Scaling (nMDS)

To visualize the rank order created by the triangular matrix of Bray-Curtis similarity values, a non-metric multidimensional scaling (nMDS) ordination was used. This nMDS was combined with the cluster analysis in order to indicate the groups marked out by the similarity cut off line and to compare the seasonal changes with the communities per site, as described in Clarke & Warwick (2001). The clusters are shown as overlays on the nMDS plot and now give an accurate representation of the sample coherency.

3.1. Cobble bed profile

The general pattern of the cobble beds is a layered structure as seen in Figure 19 (sediment profile). At the surface lie the larger pebbles and cobbles with accumulations of smaller cobbles and finer sediment admixed. Beneath it is a layer of smaller cobbles and granules turning into a layer of sand, intermittently mixed with silt.

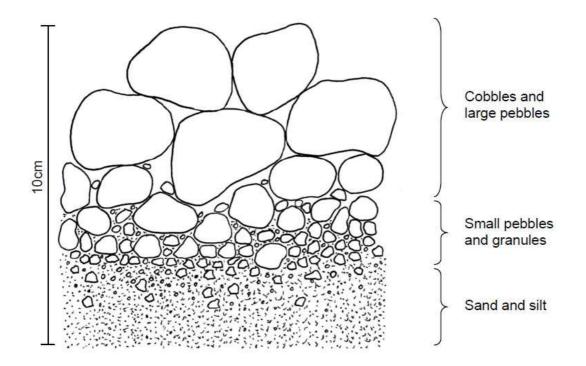


Fig. 19 Diagrammatic vertical profile of a typical cobble bed on a sediment substratum (Source: J.Evans).

3.2. Physical Characteristics

In terms of the most abundant cobble size fraction, Marsaxlokk and Tunnara were the sites with the largest mean cobble size while Hofra and Fra Ben contained the smallest cobbles with a mean size of 24 mm as seen in Table 1. Xoqqa A and Xatt I-Ahmar represent the sampling sites with an intermediate cobble size.

Sampling Site	Cobble range (mm)	Mean in mm (±SD)
Xoqqa A.	32 - 64	48 (22.6)
Marsaxlokk	64 - 128	96 (45.3)
Hofra	16 - 32	24 (11.3)
Fra Ben	16 - 32	24 (11.3)
Tunnara	64 - 128	96 (45.3)
Xatt I-Ahmar	32 - 64	48 (22.6)

Tab. 1 Results of the grain size analysis for each sampling site, showing cobble range and mean size (±SD)

3.3. Biological Characteristics

A total of 10209 individuals were collected in autumn, consisting of 14.3% molluscs and 85.7% crustaceans. In comparison, in spring only a total of 740 individuals were found, with a percentage composition of 17% molluscs and 83% crustaceans. Thus a total number of 10949 individuals were collected over both seasons with 93.2% of the biota being collected in autumn and only 6.8% in spring.

To show the variation between the sites and seasons, total abundance and species richness were used. Taken together, these parameters provide a good representation of the seasonal change.

3.3.1. Species Richness

The species richness is used to show the variation in number of species per sampling site and season. It is the count of total number of different species, without regards to the abundance or any other ecological parameters.

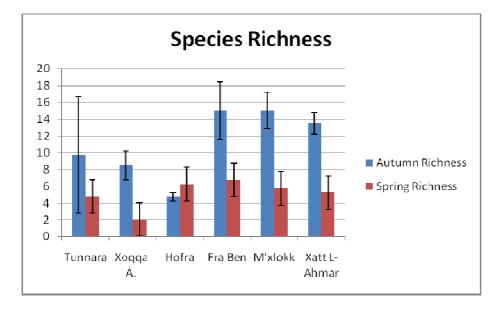


Fig.20 Mean (±SD) species richness for each sampling site per season

A total of 266 species, with an average of 11 species per site, were found in autumn. In contrast, a total of only 123 species, with an average of 5 species per site, were recorded in spring.

As seen in Fig. 20 the most distinct difference is that the species richness in autumn is generally higher compared to spring in nearly all the sites. The highest difference is seen for Marsaxlokk and Fra Ben with a reduction of more than 55 %. These two sites had the highest number of species richness in autumn.

Similarly, Xatt I-Ahmar had high species richness in autumn and registered a significant reduction in spring. Tunnara and Xoqqa A. bear a resemblance in species richness in autumn but experience a different reduction of approximately 50% for Tunnara and more than 65% for Xoqqa A. in spring. Hofra did not show the same pattern as the other sites, since in this case the mean number of species increased slightly from an average of approximately 5 species per sample in autumn to a value of 6 species per sample in spring.

Two-way ANOVA

Two-way ANOVA was used to test if there were any statistically significant differences in species richness between the different sampling sites and seasons.

Tab. 2 results of the two-way ANOVA test in species richness between sampling sites (Si) and seasons (SE)

```
Experimental Details
Number of factors: 2
Factor 1 is Season has 2 levels is orthogonal and is fixed
Factor 2 is Site has 6 levels is orthogonal and is fixed
Number of replicates: 4
Transform: Ln(X+1)
Cochran's Test
C = 0.3774 (P < 0.05)
Largest variance = 0.4605, this belongs to cell Level: 2 2
The model for this analysis is :
X = MEAN + Se + Si + SeXSi + RES
                 SS DF
                                           F
                                  MS
                                                   P F versus
Source
            5.5458 1
3.8463 5
2.4840
                              5.5458 54.54 0.0000 RES
Se
                              0.7693 7.57 0.0001 RES
Si
SeXSi
            2.4849 5
                                       4.89 0.0016 RES
                              0.4970
            3.6603 36
RES
                               0.1017
            15.5372 47
TOT
```

The results (Table 2) show that differences were in fact present between both sites (F = 7.57, p = 0.001) and seasons (F = 54.54, p < 0.001). The SNK post-hoc tests indicated that there were significant differences between the seasons at all sites.

Fra Ben, Xatt I-Ahmar and Marsaxlokk differ with higher species richness in autumn than the other sites and the heaviest decrease in spring. Hofra was significantly different during both seasons, lowest species richness in autumn but with an increase during winter.

3.3.2. Total Abundance

Total abundance is used to show the change in the number of individuals between the seasons and sites. It shows the total amount of all individuals irrespective of species in that specific sample.

The mean total abundance for autumn was 425.3 individuals per sample, during the winter month the number dropped to a mean of 30.875 individuals per sample.

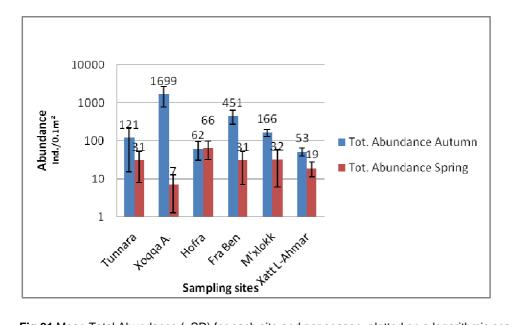


Fig.21 Mean Total Abundance (±SD) for each site and per season, plotted on a logarithmic scale.

Figure 21 shows the total abundance between the seasons for each site. There is a great fluctuation not just between the sites, but also between the seasons. This is evident for Xoqqa A., were the abundance decreased by 99.6%, from more than 1000 to less than 10.

Another significant decrease occurred at Fra Ben, also by more than 90%. A slightly lower reduction in abundance took place in Marsaxlokk and Tunnara, roughly 75-80% from autumn to spring. On the contrary, Hofra experienced an increase of around 7%.

Two-way ANOVA

Two-way ANOVA was used to test if there were any statistically significant differences in total abundance between the different sampling sites and seasons.

Tab. 3 results of the two-way ANOVA test in total abundance between sampling sites (Si) and seasons (SE)

```
Experimental Details
Number of factors: 2
Factor 1 is Season has 2 levels is orthogonal and is fixed
Factor 2 is Site has 6 levels is orthogonal and is fixed
Number of replicates: 4
Transform: Ln(X+1)
Cochran's Test
C = 0.2944 (Not Significant)
Largest variance = 1.4224, this belongs to cell Level: 2 2
The model for this analysis is :
X = MEAN + Se + Si + SeXSi + RES
Source
                   SS DF
                                       MS
                                                F
                                                         P F versus
              52.5386 1 52.5386 130.51 0.0000 RES
7.3838 5 1.4768 3.67 0.0087 RES
Se
            52.5386
                                1.4768 3.67 0.0087 RES
7.6667 19.04 0.0000 RES
Si
             38.3333 5
14.4926 36
SeXSi
RES
                                  0.4026
TOT
            112.7484
                       47
```

The results (Table 3) show that differences were in fact present between both sites (F = 3.67, p = 0.009) and seasons (F = 130.5, p < 0.001). The SNK post-hoc tests indicated that there were significant differences between seasons at all sites except for Hofra. In all cases, these differences were due to a higher total abundance being recorded in autumn. For the autumn sampling session, Xoqqa A. and Fra Ben differed significantly from all the other sites, with Xoqqa A. repeatedly having the highest mean total abundance and Fra Ben the second highest mean total abundance.

In spring, Xoqqa A. was also significantly different from all other sites, but in this case it had the lowest total abundance since it experienced the highest decrease in mean total abundance overall.

3.4. Multivariate Analysis

A cluster analysis was carried out to subdivide the data into similar "natural" groups and reveal associations, patterns, relationships and structures in the data. SIMPROF permutation tests were accomplished in order to show whether or not there were indicative differences between the clusters. The black lines correspond to a significant difference and red lines indicate there is not. SIMPROF identified 5 clusters, which were reduced to 4 clusters by taking a similarity cut-off at a slightly higher level.

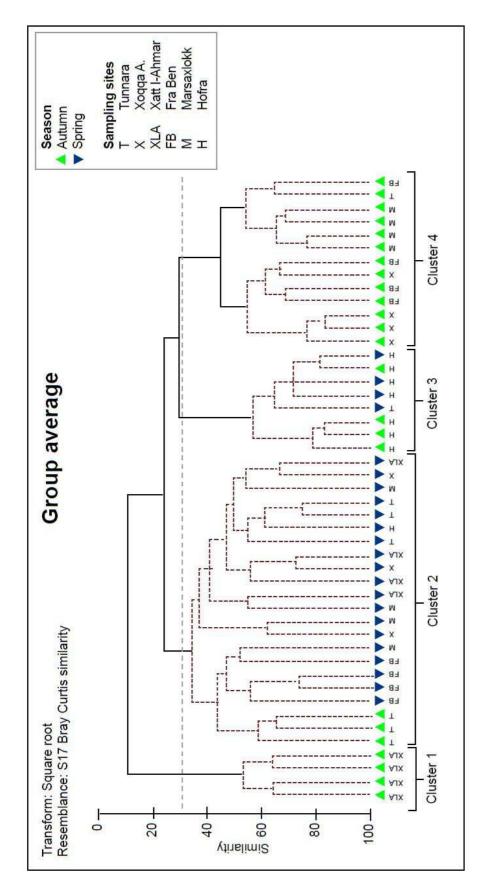


Fig.22 Dendrogram resulting from agglomerative group average linkage, hierarchical clustering of the samples based on the mean abundance for each species with the results of the SIMPROF procedure marked in red; the similarity cut off is at 30%.

The single clusters, identified by the cluster analysis were composed as follows:

Cluster 1	A Xatt I-Ahmar	nmar A Xatt L-Ahmar2 A Xatt L-Ahmar		A Xatt L-Ahmar
	1		3	4
Cluster 2	A Tunnara 1 S	S Fra Ben 2	A Tunnara 3	A Tunnara 4
	Xoqqa A. 1	S Marsaxlokk 2	S Fra Ben 3	S Fra Ben 4
	S Xatt I-Ahmar 1	S Xoqqa A.2	S Marsaxlokk 3	S Marsaxlokk 4
	S Fra Ben 1	S Xatt I-Ahmar 2	S Xatt I-Ahmar 3	S Xoqqa A. 4
	S Marsaxlokk 1			S Hofra 4
Cluster 3	A Hofra 1	A Hofra 2	A Hofra 3	A Hofra 4
	S Hofra 1	S Hofra 2	S Hofra 3	A Tunnara 4
Cluster 4	A Fra Ben 1	A Fra Ben 2	A Fra Ben 3	A Fra Ben 4
	A Marsaxlokk 1	A Marsaxlokk 2	A Marsaxlokk 3	A Marsaxlokk 4
	A Xoqqa A. 1	A Xoqqa A. 2	A Xoqqa A. 3	A Xoqqa A. 4
				A Tunnara 4

Tab.4. The four clusters identified from the cluster analysis

<u>nMDS</u>

nMDS was carried out to visually represent the rank order within the underlying similarity matrix, by emphasis on the relative distance between points, with the stress value of 0.17. A stress value serves as the indicator of 'quality' of the ordination plot. A value between 0.1 and 0.2 provides accurate results for interpretation.

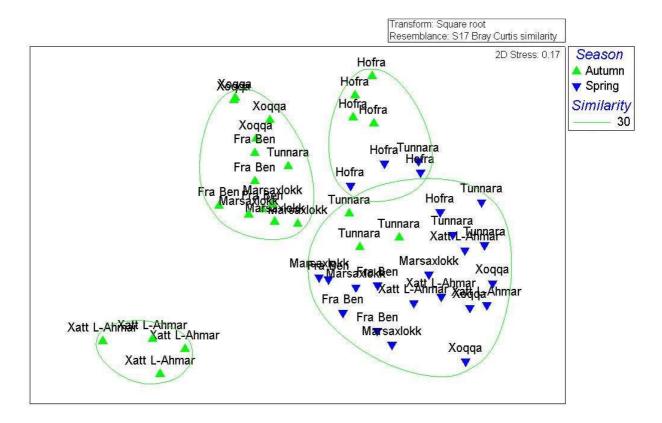


Fig.23 nMDS plot for the samples based on the mean abundance for each species and using the Bray-Curtis coefficient as a similarity measure. The four clusters resulting from cluster analysis at a similarity cut off of 30% are indicated as bubble overlays

The nMDS ordination plot (Fig. 23) shows that the cluster of Xatt I-Ahmar autumn (Cluster 1) is far different from all of the other samples. The other samples are more similar to each other, but divide into three separate groups. Hofra forms a group containing all samples from autumn and spring (Cluster 3). All of the remaining autumn samples, except Tunnara, form another group (Cluster 4) and the final group contains all remaining spring samples and also includes one sample of Tunnara autumn (Cluster 2).

<u>SIMPER</u>

SIMPER analysis was carried out to detect the main species responsible for the clustering patterns observed. The results are shown in Tables 5 - 7; only species contributing up to 90% of the total similarity/dissimilarity are included.

The results of the SIMPER analysis indicated the species composition across all samples as fairly similar except for Cluster 1, which shows significant differences in composition and average abundance of the single species. While most of the clusters are dominated by typical species such as *Gibbula varia*, *Melita hergensis* and *Gammarella fucicola*, Cluster 1 is dominated by species such as *Leptochelia savignyi* and *Ischnochiton rossoi*, which do not appear in any of the other clusters. These two species contribute to approximately 70% of the total abundance.

Tab.5 SIMPER analysis of similarity for cluster 1 based on the clusters identified by SIMPROF

Cluster 1							
Average similarity: 62.38							
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%		
Leptochelia savignyi	24.00	36.71	6.26	58.86	58.86		
Xantho pilipes	5.25	7.50	2.39	12.02	70.88		
Ischnochiton rissoi	5.25	6.99	1.97	11.21	82.10		
Bittium latreillii	3.50	5.00	4.64	8.02	90.11		

Cluster 3 is dominated by the crustacean species *Paryhale aquilani* and the mollusc complex *Gibbula divaricata /rarilineata*. *Melita hergensis*, a common and high abundant species in the other clusters (Tab 7.) shows a remarkable low abundance and contribution in this cluster.

Tab. 6 SIMPER analysis of similarity for cluster 3, based on the clusters identified by SIMPROF

Cluster 3						
Average similarity: 54.25						
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%	
Parhyale aquilina	37.63	30.92	1.87	57.00	57.00	
Gibbula divaricata/ rarilineata	16.13	15.34	1.22	28.28	85.28	
Melita hergensis	7.63	3.37	0.55	6.21	91.49	

However, due to its seasonal differences cluster 2 and 4 present differences in the species composition and average abundance. The most abundant species common in both clusters, *Gammarella fucicola* and *Melita hergensis*, show a remarkable lower average abundance in cluster 2 compared to what was observed in cluster 4. Similar reduction can be seen on other species such as *Paryhale aquilani* and *Gibbula divaricata/ rarilineata* complex.

Nevertheless, opposite to the previous described scenario, *Gibbula varia* and *Phorcus richardi* show a significant increase in their average abundances in cluster 2. A slight increase was also observed on *Clibanarius erythropus*.

Cluster 2 & 4						
Average dissimilarity = 75.23						
Average dissimilativy = 75.25						
	Cluster 2	2 Cluster 4	Ł			
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Gammarella fucicola	47.67	237.41	21.05	0.86	27.98	27.98
Melita hergensis	30.33	109.41	16.31	1.57	21.68	49.66
Gibbula varia	32.06	20.47	13.04	0.99	17.33	66.99
Gibbula divaricata/ rarilineata	1.44	16.94	6.09	0.62	8.10	75.08
Parhyale aquilina	0.94	69.06	5.71	0.66	7.60	82.68
Phorcus richardi	2.39	1.00	2.19	0.34	2.91	85.59
Clibanarius erythropus	1.83	1.76	1.71	0.45	2.28	87.86
Leptochelia savignyi	0.72	1.41	0.87	0.65	1.16	89.02
Xantho pilipes	0.39	2.59	0.80	0.73	1.06	90.08

Tab. 7 SIMPER analysis for dissimilarity of Cluster 2 & 4, based on the clusters identified by SIMPROF

4.1. Context

The results obtained in the present study into the effects of seasonal change on the cobble bed habitats indicated that there is a distinct difference between seasons in the quantitative (abundance) and qualitative (species composition) parameters. As shall be discussed in the following sections, there is not just a change between the seasons, but significant differences between sampling sites with similar conditions have also been discovered.

As seen in the dendrogram (Fig. 22), the two seasons differ significantly in terms of total abundance, species richness and composition, forming two different clusters. Osman (1977) noted the same effect during the examination of marine epifaunal communities living on rocks, and stated that there is a seasonal change in the number of sessile invertebrate species, varying from a high number at the end of summer to a low number at the end of winter. In the present study, although the numbers change, the relative abundance of molluscs and crustaceans recorded within a season remained at the same proportions. This corresponds with the results of Linnane et al. (2011) where crustaceans were the most numerically abundant group within cobble sites, and thus appear to have a close association with this shelter-providing habitat.

The same effect is seen when comparing the SIMPER results, which showed a heavy decrease in total abundance of crustacean species between autumn and spring. Comparing the abundance of mollusc species, one conspicuous difference is that some species like *Gibbula varia* and *Phorcus richardi* bear a slight increase in abundance during the winter months, as does the hermit crab *Clibanarius erythrophus*. An explanation for that could be the protective property of the shell, protecting these species from mechanical damage by the moving cobbles. The increase could be a consequence of less competition for food. It is necessary to conduct a study on these particular species, their feeding type and the general level of competition between the species in order to test this hypothesis.

The seasonal change can also be observed in the total species richness per site (across all samples), with usually more than ten species per site in autumn and a peak of only seven in spring; comparing the average richness gives the same result of decreasing numbers in spring. Osman (1977) explained such decrease in species richness by stating that just a few species remain in cobble habitats constantly, while several species appear to disappear following disturbance. In addition, Robinson & Tully (2000) noted that several species settle into these habitats but fail to persist until the end of the season.

The results of the SIMPER tests affirm that statements, showing that the indicator species for cobble habitats defined by Peres (1967), like *Melita hergensis* or *Paryhale aquilina* remain, but undergo a decrease in number of individuals, while several other species like *Ischnochiton rissoi* disappear completely during the winter. Future studies on these particular species could reveal their mechanisms for surviving, that is, how they escape being damaged by the moving cobbles and how they deal with the harsh environment.

Lieberman et al. (1979) noted significant seasonal variations of cobble colonisation between the calm period and the rainy season in Ghana. They also found differences between colonisation in relation to the cobble size and their tumbling rate. In the present study, no difference in species richness comparing to cobble size was found. This is clear from the data for Marsaxlokk, Fra Ben and Xatt I-Ahmar; these three sites have different cobble size distributions, but have similar high species richness, as well as a similar decrease in richness in spring. There is also no species change regarding the different cobble size; the most abundant species were the same in all the various samples irrespective of cobble size distribution. Another factor of disturbance which Lieberman et al. (1979) mentioned is the tumbling rate, which is correlated to the exposure of the sites. In the present study all of the sampling sites have approximately the same level of exposure, and therefore are presumed to be subject to the same levels of storm and wave activity.

As seen in the dendrogram (Fig.22), Xatt I-Ahmar in autumn forms a cluster (cluster 1) completely different from all the other sites, while in spring no difference was identified. The difference between this site and the others was mainly due to species composition. While at the other sites the fauna was dominated by the same species, at Xatt I-Ahmar it was significantly different and was dominated by different species. These differences might have something to do with the fact that the cobble bed at Xatt I-Ahmar is slightly deeper than the others. Also, Xatt I-Ahmar is surrounded by rocky shores with a steep slope and there is no shingle beach, which means that there is no shallow water zone adjacent to the shore. These conditions may protect the biota from any disturbance during the calm season as well as from trampling by swimmers or other man-made disturbances. However, the depth of the cobble bed appears to make a difference only in the calm summer season; during the winter it seems not to be deep enough to modulate the effects of disturbance due to storm activity that would result in significant differences in species composition. Future studies on the relationships between cobble beds at different depths and the intensity of disturbance could are needed to test this hypothesis.

Like Xatt I-Ahmar, Hofra samples form another cluster by themselves in autumn and spring, but the main difference here is that in contrast to all sites, II-Hofra z-Zghira shows an increase in total abundance and species richness during the winter which is opposite to what other studies have found; for example, Osman (1977), who assumed that the settling process happens in spring, with the main growing process during the summer months and a minimum growth occurrence during the winter months.

According to Zimmerman et al. (1979), *Posidonia oceanica* detritus is an important food supply for gammaridean amphipods. In this context, while investigating the effect of the warm water outflow from the Delimara Power Station on *Posidonia oceanica*, Gatt (2006) noted that the temperature in the shallow water parts of Hofra is significantly higher than that within the same depth zone at the reference site. Alcoverro et al. (1995) showed that the main growing season of *Posidonia oceanica* depends mainly on the light-temperature variation; they observed that the two main leaf- loss periods of shallow water meadows appear during summer, and due to the higher disturbance, in mid-winter. The close proximity to *Posidonia oceanica* meadows and the constant availability of food sources with presumably little seasonal fluctuations could be the reason for the steady number of individuals in Hofra throughout the year, while recruitment or immigration could be the reason for the slight increase. Further studies in this particular area on the feeding type and food supply of the cobble bed biota are necessary to in order to test this hypothesis.

4.1. Conclusion

The present study has shown that there is a pronounced effect of different levels of disturbance linked to the seasonal change on cobble beds and the biota inhabiting them. Most of the highly abundant species in autumn, such as Gibbula divaricata/ rarilineata and Gammarella fucicola show a large decrease in individuals during winterand other species disappear completely. However, there are also species that manage to survive throughout the whole year in good abundances; these include *Melita hergensis* and *Parhyale aquilina* with only minor fluctuations in abundance within the seasonal change. These species have presumably different survival mechanisms to deal with these dynamic conditions; however, to reveal these strategies it is necessary to conduct a study on these particular species, focusing on seasonal differences, which was beyond the scope of the present work. There are also species which seem to benefit from these seasonal changes, using special protection techniques to avoid mechanical damage by tumbling cobbles for example, Gibbula varia or Clybanarius erythropus both of which are protected by tough shells. These species are, compared to other species, less abundant throughout the whole year but seem to exploit the winter to increase their number while other species are less abundant or absent altogether. Further studies are necessary to corroborate this hypothesis.

All the observations made in the present study support the hypothesis that the higher disturbance caused by waves during the winter season causes a significant change to most of the animal populations of cobble bed habitats but it was not observed if the numbers change because of mortality of the biota or because of movement to deeper bottoms during the winter season in order to escape the disturbance, with a return migration back as soon as the calm season begins (Peres 1967)). It is necessary to conduct a study on migration patterns between cobble bed habitats at different depths in order to verify this hypothesis.

Chapter 1: Introduction

Chapter 2: Materials and Methods

Chapter 3: Results

Chapter 4: Discussion

References

- Alcoverro, T.; Duarte, C.M.; Romero, J. (1995), Annual growth dynamics of Posidonia oceanica: contribution of large-scale versus local factors to seasonality, Marine Ecology Progress Series Vol. 120: 203-210. 1995
- Cachia, C.; Mifsud, C. (1996), *The marine Mollusca of the Maltese Islands*. Leiden: Backhuys Publishers,
- Clarke, K. R., and Warwick, R.M. (1994), *Change in marine communities: an approach to statistical analysis and interpretation*. Plymouth: Natural Environment Research Council, 1994.
- Clarke, K. R.; Gorley, R. N. (2006), *Primer v6: user manual tutorial*. Plymouth: Plymouth Marine Laboratory
- Clarke, R. K.; Somerfield, P.J.; Gorley, R.N. (2008), Testing of null hypotheses in exploratory community analyses: similarity profiles and biota-environment linkage, Journal of Experimental Marine Biology and Ecology 366 56–69
- Cowardin, L. M.; Carter, V.; Golet, F. C.; LaRoe, E. T.(1979), *Classification of wetlands and deepwater habitats of the United States*, U.S. Department of the Interior, Fish and Wildlife Service, Office of Biological Services, Washington, D.C.
- Cutajar, D.; Buhagiar, A.; Schembri, P.J.; Ventura, F.: *The climate of the Maltese Islands. A review.* http://www.shadowservices.com/nature/Maltese/Geomorph/climate.htm retrieved on 26/06/2013
- Davies, A.; Wilce, R. (1987), Algal diversity in relation to physical disturbance a mosaic of successional stages in a subtidal cobble habitat, Marine Ecology Progress Series Vol. 37: 229-237.
- Davies, A.N.; Wilce, R.T. (1987), *Floristics, phonology, and ecology of the sublittoral marine algae in an unstable cobble habitat,* Phycologia Vol.26 (1), pp.23-34
- Deidun, A.; Azzopardi, M.; Saliba, S. & Schembri, P.J. (2003), Low faunal diversity on Maltese sandy beaches: fact or artefact? Estuarine, Coastal and Shelf Science 58 (supplement 1): 83-92.

- Evans, J. (2007), *Characteristics of an infralittoral cobble beds at Sliema Creek with special reference to the molluscan assemblages*, Unpublished BSc dissertation, Department of Biology, University of Malta.
- Falciai, L.; Minervini, R. (1992), Guida dei crostacei decapodi d' Europa. Padova: Muzzio,
- Gatt, M. (2006), Effects of the thermal effluent from the Delimara Powerstation (Malta) on the Seagrass Posidonia oceanica, Unpublished MSc dissertation, Department of Biology, University of Malta.
- Gauci, M.J.; Deidun, A. & Schembri, P.J. (2005) Faunistic diversity of Maltese pocket sandy and shingle beaches: are these of conservation value? Oceanologia 47 (2): 219-241.
- Gofas, S. (2011), *Moluscos marinos de Andalucía*. Malaga: Universidad de Málaga, Servicio de publicaciones e intercambio científico
- Hayward, P. J.(1995), *Handbook of the marine fauna of North-West Europe*. Oxford: Oxford university press.
- Lieberman, M. And D.; John, D.M.(1979) *Ecology of subtidal algae on seasonally devastated cobble substrates off Ghana*, Ecology, Vol.60 No.6, pp. 1151-1161
- Irving, A.D.; Bertness, M.D. (2009), *Trait-dependent modification of facilitation on cobble beaches*, Ecology, 90(11), pp. 3042–3050
- Linnane, A.; Ball, B.; Mercer, J.P.; Browne, R.; van der Meeren, G.; Ringvold, H.; Bannister, C.; Mazzoni, D. and Munday, B. (2001), Searching for the early benthic phase (EBP) of the European lobster - a trans-European study of cobble fauna, Hydrobiologia 465: 63-72
- Linnane,A.; Ball, B.; Munday,B. ;Browne R.; Mercer, J.P.(2003), *Faunal description of* an irish cobble site using airlift suction sampling, Biology and Environment: Proceedings of the Royal Irish Academy, Vol. 103B, No. 1, 41–48 (2003).

- Manousis, T. (2012), *The sea shells of Greece: 1200 species 1800 illoustration 180 drawing [sic]*. Thessaloniki: Kyriakidis Brothers s.a.
- McClintock, J.B. and F.E.; Angus, R.A.; (2007), Abundance, diversity and fidelity of macroinvertebrates sheltering beneath rocks during tidal emersion in an intertidal cobble field: Does the intermediate disturbance hypothesis hold for less exposed shores with smaller rocks?, Journal of Experimental Marine Biology and Ecology 352 (2007) 351–360
- Naylor, E.E. (1972), *British marine isopods: keys and notes for the identification of the species*. London: Academic Press for the Linnean Society of London.
- Osman, R. W., (1977) The establishment and development of a marine epifaunal community, Ecological Monographs 47, pp.37-63
- Robinson, M.; Tully, O. (2000), Seasonal variation in community structure and recruitment of benthic decapods in a sub-tidal cobble habitat, Marine Ecology Progress Series Vol. 206: 181–191, 2000
- Ruffo, S. (1998), *The Amphipoda of the Mediterranean*. Monaco: Institut Oceanographique.
- Scheibling, R.E.; Raymond, B.R. (1990) Community dynamics on a subtidal cobble bed following mass mortalities of sea urchins, Marine Ecology Progress Series Vol. 63: 127-145, 1990
- Scheibling, R.E, Kelly N.E.; Raymond, B. G.(2009), *Physical disturbance and community organization on a subtidal cobble bed*, Journal of Experimental Marine Biology and Ecology 368 (2009) 94–100
- Scott, G. A. M. (1963), *Ecology of Shingle Beach Plants*, Journal of Ecology, Vol. 51, No. 3 (Nov., 1963), pp. 517-527
- Sousa, W.P. (1979) Disturbance in marine intertidal boulder fields: The Nonequilibrium Maintanace of Species Diversity, Ecology, Vol. 60, No.6 (1979), pp.1225-1239

- Van de Koppel, J.; Altieri, A.H.; Silliman, B.R.; Bruno, J.F.; Bertness, M.D. (2006), -Dependent interactions and community structure on cobble beaches, Ecology Letters, (2006) 9: 45–50
- Villora-Moreno, S., Capaccioni-Azzati, R. and Garcia-Carrascosa, A. M.(1991), Meiobenthos of sandy beaches from the Gulf of Valencia (Western Mediterranean): Ecology of interstitial Polychaetes, Bulletin of Marine Science, 48(1991): 376-385,
- Zimmerman,R.; R. Gibson, J. Harrington. *Herbivory and detritivory among gammaridean amphipods from a Florida seagrass community*. Marine Biology September (I) 1979, Volume 54, Issue 1, pp 41-47

<u>ii. Appendix</u>

Tab. A. Results of the SIMPER analysis with the factor "cluster", identified by SIMPROF

Tab. A. 1.

Cluster 2					
Average simil	arity: 27.97				
Species	Av, Abund	Av.Sim	Sim/SD	Contrib%	Cum. %

Melita hergensis30.3321.881.1878.2278.22Gibbula varia32.063.520.4912.6090.82	species	Av.Abuna	AV.Sim	SIM/SD	CONTLID%	Cum. 8
Gibbula varia 32.06 3.52 0.49 12.60 90.82	Melita hergensis	30.33	21.88	1.18	78.22	78.22
	Gibbula varia	32.06	3.52	0.49	12.60	90.82

A . 2.

Cluster 4					
Average similarity: 24.62					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Melita hergensis	109.41	13.11	0.95	53.25	53.25
Gibbula varia	20.47	4.89	0.70	19.86	73.10
Gammarella fucicola	237.41	2.76	0.32	11.19	84.29
Gibbula divaricata/ rarilineata	16.94	1.79	0.35	7.29	91.58

Tab. B results of the SIMPER analysis of dissimilarity, with the factor "cluster", identified by SIMPROF

В. 1.

Average dissimilarity = 85.60							
	Group 2	Group 3					
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Av.Abund Av.Abund Av.Diss Diss/SD Contrib% Cum.%	Cum.%	
Parhyale aquilina	0.94	37.63	29.65	1.44	34.64	34.64 34.64	
Gibbula divaricata/ rarilineata	1.44	16.13	14.80	0.99	17.28	51.93	
Melita hergensis	30.33	7.63	12.35	1.41	14.43	66.36	
Gibbula varia	32.06	3.88	11.22	0.86	13.11	79.46	
Gammarella fucicola	47.67	0.88	8.88	0.52	10.37	89.84	
Phorcus richardi	2.39	0.00	1.89	0.31	2.21	92.05	

B. 2.

Cluster 4 & 3 Average dissimilarity = 81.29

	Group 4	Group 3				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Av.Abund Av.Abund Av.Diss Diss/SD Contrib% Cum.%	Cum. %
Parhyale aquilina	69.06	37.63	24.24	1.36	29.82	29.82 29.82
Gammarella fucicola	237.41	0.88	16.40	0.69	20.17	20.17 49.99
Melita hergensis	109.41	7.63	14.25	1.70	17.53	17.53 67.52
Gibbula divaricata/ rarilineata	16.94	16.13	12.29	0.94	15.12	15.12 82.64
Gibbula varia	20.47	3.88	6.73	1.10	8.28	8.28 90.93

Average dissimilarity = 96.44							
	Group 2	Group 1					
Species	Av.Abund	Av. Abund Av. Diss	Av.Diss	Diss/SD	Contrib% Cum.%	Cum.%	
Leptochelia savignyi	0.72	24.00	24.72	1.79	25.63	25.63	
Melita hergensis	30.33	00.00	16.18	1.93	16.78	42.42	
Gibbula varia	32.06	1.25	11.01	0.75	11.42	53.83	
Gammarella fucicola	47.67	0.50	8.97	0.51	9.30	63.14	
Ischnochiton rissoi	00.00	5.25	5.53	1.47	5.73	68.87	
Xantho pilipes	0.39	5.25	5.48	1.31	5.68	74.55	
Bittium latreillii	0.00	3.50	3.66	1.64	3.79	78.34	
Clibanarius erythropus	1.83	1.25	2.12	0.64	2.20	80.54	
Phorcus richardi	2.39	00.00	2.11	0.32	2.18	82.72	
Calcinus tubularis	0.06	2.00	1.92	1.01	1.99	84.71	
Maera grossimana	0.22	1.25	1.25	1.13	1.30	86.01	
Gibbula divaricata/ rarilineata	1.44	0.25	1.11	0.51	1.15	87.16	
Athanas nitescens	0.28	1.00	0.96	0.76	0.99	88.15	
Cymodoce truncata	0.11	1.00	0.91	0.73	0.95	89.10	
Dynamene spp.	0.83	0.25	0.83	0.60	0.86	89.96	
Cestopagurus timidus	00.00	0.75	0.82	1.15	0.85	90.80	

B.3.

Cluster 2 & 1

Cluster 4 & 1							
Average dissimilarity = 94.53							
	Group 4	Group 1					
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum. %	
Melita hergensis	109.41	00.00	17.79	2.32	18.82	18.82	
Leptochelia savignyi	1.41	24.00	17.28	1.23	18.27	37.09	
Gammarella fucicola	237.41	0.50	16.60	0.68	17.57	54.66	
Gibbula varia	20.47	1.25	7.82	1.09	8.27	62.93	
Gibbula divaricata/ rarilineata	16.94	0.25	5.94	0.63	6.29	69.22	
Parhyale aquilina	69.06	00.00	5.33	0.61	5.64	74.86	
Ischnochiton rissoi	00.00	5.25	3.99	1.13	4.22	79.08	
Xantho pilipes	2.59	5.25	3.73	0.92	3.94	83.02	
Bittium latreillii	00.00	3.50	2.64	1.22	2.79	85.81	
Calcinus tubularis	0.06	2.00	1.41	0.84	1.49	87.30	
Clibanarius erythropus	1.76	1.25	1.40	0.81	1.48	88.78	
Maera grossimana	0.76	1.25	0.81	0.91	0.86	89.64	
Cymodoce truncata	0.29	1.00	0.69	0.66	0.73	90.37	

B.4.

Average dissimilarity = 95.56						
	Group 3	3 Group 1				
Species	Av.Abund	Av.Abund Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum. %
Parhyale aquilina	37.63	00.00	28.96	1.97	30.30	30.30
Leptochelia savignyi	0.00	24.00	20.35	3.48	21.29	51.59
Gibbula divaricata/ rarilineata	16.13	0.25	13.97	1.39	14.62	66.21
Melita hergensis	7.63	0.00	5.80	0.97	6.07	72.28
Ischnochiton rissoi	00.00	5.25	4.48	2.23	4.69	76.97
Xantho pilipes	1.38	5.25	3.49	1.51	3.66	80.63
Gibbula varia	3.88	1.25	3.22	1.11	3.37	84.00
Bittium latreillii	00.00	3.50	2.98	2.70	3.12	87.12
Calcinus tubularis	00.00	2.00	1.62	1.23	1.70	88.81
Clibanarius erythropus	0.13	1.25	1.05	1.02	1.10	89.91
Maera grossimana	0.00	1.25	1.05	1.47	1.09	91.01

B. 5.

Cluster 3 & 1

Tab. C results of the SIMPER analysis with the factor "season"

C. 1.

Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%	
37.04	5.40	0.76	25.15	25.15	
90.25	4.35	0.67	20.25	45.40	
16.79	3.86	0.49	18.00	63.40	
203.67	3.71	0.39	17.30	80.70	
5.38	1.25	0.23	5.83	86.53	
54.04	1.03	0.27	4.79	91.32	
	37.04 90.25 16.79 203.67 5.38	37.045.4090.254.3516.793.86203.673.715.381.25	37.045.400.7690.254.350.6716.793.860.49203.673.710.395.381.250.23	37.045.400.7625.1590.254.350.6720.2516.793.860.4918.00203.673.710.3917.305.381.250.235.83	90.254.350.6720.2545.4016.793.860.4918.0063.40203.673.710.3917.3080.705.381.250.235.8386.53

C. 2.

Group Spring						
Average similarity	7: 34.94					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%	
Melita hergensis	12.54	28.51	1.54	81.60	81.60	
Gibbula varia	3.00	2.64	0.52	7.57	89.16	
Parhyale aquilina	8.13	1.92	0.25	5.50	94.66	

Tab. D results of the SIMPER analysis with the factor "site".

D. 1.

Group Hofra					
Average similarity: 59.34					
				<i>~</i>	~ ^
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Gibbula divaricata/ rarilineata	23.25	32.60	3.13	54.93	54.93
Parhyale aquilina	30.75	17.91	2.46	30.18	85.12
Gibbula varia	5.75	6.80	1.36	11.45	96.57

D. 2.

Group Tunnara	
Average similarity: 41.2	2

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Gibbula varia	42.25	24.16	1.48	58.62	58.62
Melita hergensis	29.25	11.57	2.60	28.07	86.69

D.3.

Group Xoqqa A.					
Average similarity:	57.97				
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Gammarella fucicola	978.75	39.61	3.60	68.34	68.34
Melita hergensis	377.75	9.62	1.85	16.59	84.92

D. 4.

Group Fra Ben						
Average similarity:	52.68					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%	
Gammarella fucicola	212.50	21.30	1.16	40.44	40.44	
Melita hergensis	91.75	14.92	2.18	28.31	68.75	
Gibbula varia	117.50	14.63	1.23	27.78	96.53	

D.5.

Group Marsaxlokk Average similarity: 67.61

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Gibbula divaricata/ rarilineata	44.25	22.71	2.78	33.60	33.60
Melita hergensis	42.25	17.04	1.96	25.20	58.80
Gibbula varia	26.00	14.51	5.13	21.46	80.26

D.6.

Ischnochiton rissoi

Group Xatt L-Ahmar					
Average similarity: 6	2.38				
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Leptochelia savignyi	24.00	36.71	6.26	58.86	58.86
Xantho pilipes	5.25	7.50	2.39	12.02	70.88

1.97

11.21 82.10

5.25

6.99

Tab .E results of the SIMPER analysis of dissimilarity, with the factor "season"

Е. -

Groups Autumn & Spring						
Average dissimilarity = 87.99						
	Group Autumn	Group Autumn Group Spring				
Species	Av. Abund	Av.Abund	Av.Diss	Diss/SD	Diss/SD Contrib%	Cum. %
Gammarella fucicola	203.67	0.63	18.69	0.76	21.24	21.24
Melita hergensis	90.25	12.54	15.28	1.64	17.36	38.60
Gibbula varia	37.04	3.00	13.10	06.0	14.89	53.49
Parhyale aquilina	54.04	8.13	10.77	0.71	12.24	65.73
Gibbula divaricata/ rarilineata	16.79	1.71	10.16	0.73	11.55	77.28
Leptochelia savignyi	5.38	0.17	5.71	0.48	6.49	83.77
Phorcus richardi	2.17	0.33	2.12	0.27	2.41	86.18
Xantho pilipes	3.08	0.38	1.98	0.68	2.25	88.42
Clibanarius erythropus	1.83	1.04	1.32	0.52	1.50	89.92
Ischnochiton rissoi	0.88	00.00	1.14	0.39	1.29	91.22

Tab. F Results of the two-way ANOVA test on species Richness, with the factor season (SE) and site (SI)

F.1.

```
Header File: None
Data File : D:\BA\Analysis\Sp Richness.txt
Transform: Ln(X+1)
Factor : Se
               S.E. for comparison : 0.0651
Levels : -
Cell :
Mean :
S.E. :
                    1
                                 2
                         1.7241
0.0997
               2.4039
               0.0903
Rank 2 - 1
Cell 1 - 2
           * *
Factor : Si
               S.E. for comparison : 0.1127
Levels : -
                       2 3
1.5957 1.8447
0.2924 0.0822
Cell :
Mean :
                    1
                                                           4
                                                   2.3932
0.1536
               1.9869
                                                                  2.3277
     :
S.E.
               0.1712
                                                                  0.1769
Cell :
                    6
Mean : 2.2360
S.E. : 0.1798

  \begin{array}{r}
    6 & - & 1 \\
    4 & - & 2 \\
    * *
  \end{array}

Rank
Cell
        Rank
Cell
        6 - 3
4 - 1
Rank
Cell
           **
                              NS
                       *
      3 - 1 4 - 2 5 - 3
1 - 2 6 - 3 5 - 1
Rank
                                      6 - 4
Cell
                                      4 - 6
                       *
                              NS
                                        NS
               Rank
        2 - 1
                             4 - 3
                                        5 - 4
                                                  6 - 5
        3 - 2
                                        5 - 6
                                                 4 - 5
Cell
                   NS
         NS
                              NS
                                        NS
                                                    NS
```

F. 2.

```
Factor : Se(Si) S.E. for comparison: 0.1595
Levels : 1
Cell : 1 2
Mean : 2.2449 1.7289
S.E. : 0.2793 0.1199
Rank 2 - 1
Cell 1 - 2
               *
Factor : Se(Si) S.E. for comparison: 0.1595
Levels : 2

      Cell :
      1
      2

      Mean :
      2.2397
      0.9517

      S.E. :
      0.0863
      0.3393

Rank 2 - 1
Cell 1 - 2
**
Factor : Se(Si) S.E. for comparison: 0.1595
Levels : 3
Cell : 1 2
Mean : 1.7462 1.9433
S.E. : 0.0456 0.1515
Rank 2 - 1
Cell 2 - 1
NS
Factor : Se(Si) S.E. for comparison: 0.1595
Levels : 4
Cell : 1 2
Mean : 2.7564 2.0299
S.E. : 0.1018 0.1082
Rank 2 - 1
Cell 1 - 2
**
Factor : Se(Si) S.E. for comparison: 0.1595
Levels : 5
Cell : 1 2
Mean : 2.7653 1.8900
S.E. : 0.0710 0.1155
Rank 2 - 1
Cell 1 - 2
**
```

Tab G. Results of the two-way ANOVA test on total abundance, with the factors season (SE) and site (SI)

G. 1.

```
Header File: None
Data File : D:\BA\Analysis\Tot Ab.txt
Transform; Ln(X+1)
Factor : Se S.E. for comparison : 0.1295
Levels : -
                       1
                                        2
Cell :
                                3.0887
0.2023
                  5,1812
Mean :
                  0.2611
S.E. :
Rank 2 - 1
Cell 1 - 2
               **
Factor : Si S.E. for comparison : 0.2243
Levels : -

        Cell
        1
        2
        3
        4
        5

        Mean
        :
        3.9374
        4.5123
        4.0705
        4.6626
        4.1686

        S.E.
        :
        0.3278
        1.1038
        0.1763
        0.5580
        0.4075

Cell :
Mean :
S.E. :
               3.4583
0.2172
        6 - 1
4 - 6
Rank
Cell
Rank 5 - 1 6 - 2
Cell 2 - 6 4 - 1
                          NS
                - 38
        4 - 1 5 - 2 6 - 3
5 - 6 2 - 1 4 - 3
Rank
Cell
              NS
                           NS
                                          NS
                      4 - 2 5 - 3 6 - 4
5 - 1 2 - 3 4 - 5
NS NS NS
Rank
         3 - 1
         3 - 6
Cell
             NS
                     3 - 2
3 - 1
          2 - 1
                                    4 - 3 5 - 4
5 - 3 2 - 5
NS NS
                                                                 6 - 5
Rank
                                                                 4 - 2
Cell
         1 - 6
             NS
                          NS
                                                                     NS
```

G. 2.

```
Factor : Se(Si) S.E. for comparison: 0.3173
Levels : 1
Cell : 1 2
Mean : 4.5858 3.2890
S.E. : 0.3537 0.3098
Rank 2 - 1
Cell 1 - 2
             **
Factor : Se(Si) S.E. for comparison: 0.3173
Levels : 2

        Cell
        :
        1
        2

        Mean
        :
        7.3174
        1.7072

        S.E.
        :
        0.2900
        0.5963

Rank 2 - 1
Cell 1 - 2
Factor : Se(Si) S.E. for comparison: 0.3173
Levels : 3

        Cell
        :
        1
        2

        Mean
        :
        4.0622
        4.0788

        S.E.
        :
        0.2322
        0.3018

Rank 2 - 1
Cell 2 - 1
NS
Factor : Se(Si) S.E. for comparison: 0.3173
Levels : 4
Cell : 1 2
Mean : 6.0572 3.2679
S.E. : 0.1941 0.3447
Rank 2 - 1
Cell 1 - 2
**
Factor : Se(Si) S.E. for comparison: 0.3173
Levels : 5
 Cell : 1 2
Mean : 5.1014 3.2359
S.E. : 0.1053 0.4289
 Rank 2 - 1
Cell 1 - 2
```

G. 3.

Factor	: Se(Si)	S.E.	for comparison	: 0.3173		
	: 5		2			
Mean		1 5.1014	3.2359			
S.E.	8	0.1053	0.4289			
Rank	2 - 1					
Cell	1 - 2					
			for comparison	: 0.3173		
	: 6					
Cell.	÷	1	2			
s.E.		3.9628 0.1205	0.1896			
Rank Cell	$ \begin{array}{c} 2 & -1 \\ 1 & -2 \\ x \end{array} $					
Factor	: Si(Se) : 1	S.E.	for comparison	: 0.3173		
					2	-
Cell Mean	1	4.5858	7.3174	3 4.0622	6.0572	5.1014
S.E.		0.3537	0.2900	0.2322	0.1941	0.1053
Cell		6				
Mean	:	3.9628				
S.E.	:	0.1205				
	6 - 1					
Cell	2 - 6					
Rank	5 - 1	6 - 2				
Cell	4 - 6	2 - 3				
	**	* *				
Rank	4 - 1	5 - 2	6 - 3			
Cell	5 - 6 NS	4 - 3	2 - 1			
			5 - 3 6			
Cell	1 - 6 NS		4 - 1 2 **	- 5		
Rank	2 - 1	3 - 2	4 - 3 5	- 4	6 - 5	
Cell	3 - 6	1 - 3	5 - 1 4	- 5	2 - 4	
	NS	NS	NS	*	* *	*

G.	4.

Factor Levels				S	.E.	for	C	ompa	risc	n:	0	.31	.73								
Cell				1				2				22	i.					4			5
Mean	:		3,28	90		1,	7	072		4	1.0	788	1		3	.20	67	9	3	23	59
S.E.	:		0.30	98		0.	5	963		0	.3	018	3		0	.34	44	7	0	420	39
Cell				6																	
Mean			2.95																		
S.E.	•		0.18	96																	
	6	- 1																			
Cell		- 2																			
Rank	5	- 1	6	-	2																
Cell		- 2		-																	
		* *		1	NS																
Rank	4	- 1	5		2	6		- 3													
Cell	4	- 2		-	6 NS			- 5 NS													
										2											
Rank Cell		- 1			2 6			- 3 - 5			19 A										
CEII	3	**		1				NS			N										
Rank	2	- 1	3		2	4	i.	- 3		5	÷	4		б	2	5					
Cell		- 2			6			- 5		1	-	4		3							
		**			NS.			NS			N				N	IS					