HABITAT CHARACTERISATION OF INFRALITTORAL PEBBLE BEDS IN THE MALTESE ISLANDS

by

JULIAN EVANS

A thesis submitted to Plymouth University
in partial fulfilment for the degree of

DOCTOR OF PHILOSOPHY

School of Marine Science and Engineering
Marine Biology and Ecology Research Centre

October 2013

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Abstract

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Julian Evans

The Mediterranean biocoenosis of infralittoral pebbles has been poorly studied and very little information is available on the physical characteristics of pebble beds, on the diversity of the associated assemblages, on the spatial and temporal variation in assemblage structure, or on interactions between the physical and biotic components. The present study was therefore carried out to characterise pebble-bed assemblages as a first step towards understanding the ecological dynamics of these habitats. Preliminary surveys were made along the low-lying coasts of the Maltese Islands to map the occurrence of pebble-bed habitats. Fifteen locations with pebble coverage >25 m^2 were chosen for study and benthic sampling was undertaken between July–September 2011; water samples were also collected on a monthly basis. Five shallow sites were sampled for biota again at six-month intervals until April 2013. The pebble beds were characterised in terms of environmental parameters and biotic composition.

A total of 62,742 individuals belonging to 360 macrofaunal taxa were recorded (total sampling area: 16 m^2). Polychaetes, crustaceans and molluscs were the most common faunal groups. The recorded species included the endemic gastropod *Gibbula nivosa*, and the first central Mediterranean records of three gobiid species. Three distinct pebble-bed types were characterised based on physical and biological features: shallow beds occurring in rocky coves, beds found within creeks or seagrass meadows, and beds located in harbour environments; amendments to internationally used benthic habitat classification schemes have been proposed, since only a single category of pebble-bed habitats is currently recognised in these. A significant seasonal reduction in species richness and abundance was recorded from shallow sites, related to the higher level of disturbance occurring during winter storms. Analysis of diversity patterns in harbour sites indicated that a high richness per site and between-site variation in species composition led to the observed high diversity. Site richness was associated with fine-scale structural complexity, while environmental characteristics were correlated with variation in assemblage structure over a broad range of spatial scales.

These findings suggest that pebble beds have a higher conservation value than generally thought. The biologically derived habitat classification scheme and knowledge on assemblage-environment relationships derived from the present work will be useful to inform and guide management decisions concerning these pebble-bed habitats.

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Author's Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee. Work submitted for this research degree at the Plymouth University has not formed part of any other degree either at Plymouth University or at any another establishment.

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Chapter 1:

Introduction

Chapter 1

Introduction

1.1 Rationale for characterisation of benthic assemblages

An ecological perspective…

One of the major goals of ecology is to understand how species assemblages are distributed in nature, and the ways in which assemblage structure can be influenced by the abiotic environment and by interactions among species (e.g. Begon *et al*., 2006). Diamond (1975) coined the term “assembly rules” to describe the mechanisms causing distinct assemblages to arise in different sites out of a common pool of species. The composition of the “total species pool” (*sensu* Kelt *et al.*, 1995) present in a geographical region of interest depends on evolutionary and biogeographical processes (Shmida and Wilson, 1985).

The “actual species pool” (*sensu* Belyea and Lancaster, 1999) present at a particular site within the region is limited in three ways. Firstly, only those species which are able to disperse to the site will be available for colonisation. Secondly, only those species which possess traits enabling them to survive in the prevalent abiotic conditions at the site will be able to persist there (Kelt *et al.*, 1995; Belyea and Lancaster, 1999). Thus, only a subset of the total species pool has the potential to reach the focal site and persist there. Finally, this subset may be further limited through biotic interactions such as competition or predation (Belyea and Lancaster, 1999).

Interspecific dynamics do not occur independently of habitat features and dispersal constraints. For example, the order with which species colonise a site may influence the outcome of subsequent competitive interactions (Grover, 1994; Blaustein and Margalit, 1996). Likewise, the prevailing abiotic environment may alter interspecific interactions (Menge and Olson, 1990, Menge *et al.*, 2002) which may even switch from negative (e.g. competition) to positive (e.g. facilitation) interactions with increasing levels of environmental stress (Bertness *et al.*, 1999).

Species diversity at a given site, or alpha diversity (Whittaker, 1960, 1972), thus depends on the mechanisms which determine the composition of the actual species pool. These mechanisms can be broadly divided into those related to the physical environment and those associated with biotic interactions. This distinction, for instance, underlies Brown's (1981) "capacity rules" and "allocation rules" as well as May's (1984) "environmental control" and "biotic control" models. Collectively, these processes are known as "niche relations" (Shmida and Wilson, 1985). They include mechanisms that: (i) increase the resource spectrum available for different species to exploit, (ii) reduce niche breadth via increased resource partitioning, or (iii) enable greater niche overlap among species (MacArthur, 1972).

Variation in species composition among sites, or beta diversity (*sensu* Legendre *et al.*, 2005), can result from changes in the prevalent niche relations, for example, due to a change in the physical characteristics of the habitat. However, since the actual species pool present at a particular site is constrained by species dispersal potential, spatial differentiation may also occur in the absence of niche-based processes. This forms the basis of neutral theory (e.g. Hubbel, 2001), which accounts for species distribution patterns in terms of their dispersal history. Both niche and neutral models can account for the patterns of species abundance distributions found in nature (Gilbert and Lechowicz, 2004; Chishol and Pacala, 2010; Smith and Lundholm, 2010).

Species assemblage composition is not static, but also exhibits temporal variation. Two major viewpoints exist in this regard. The equilibrium model assumes that niche relations are important determinants of local assemblage composition, which remains relatively stable through time. The non-equilibrium view holds that local species assemblages are unstable, with species composition constantly changing due to stochastic or periodic disturbances (e.g. Sousa, 1979). While local extinction may occur within a patch, global richness is maintained via patch dynamics (see Picket and White, 1985). Neutral theory, with its emphasis on dispersal history rather than niche differentiation, is actually a model of unstable coexistence (Chesson, 2000).

The above discussion highlights how understanding the processes that determine spatio-temporal variation in species distribution patterns and the structure of assemblages is not an easy undertaking. Models of biotic versus environmental control, equilibrium versus non-equilibrium organisation, or niche versus neutral processes have sometimes been considered as being mutually exclusive (e.g. Sousa, 1979; May, 1984). However, assemblage composition is better construed as a product of interacting multiple causes (Hilborn and Stearns, 1992; Poff, 1997). In discussing biotic and environmental control, Quinn and Dunham (1983) suggested that it is more appropriate to consider the relative contribution of each alternative cause rather than view these as mutually exclusive. Similar arguments have since been made for both equilibrium versus non-equilibrium (Chesson and Huntly, 1997) and niche versus neutral (Chave, 2004; Leibold and McPeek, 2006) processes.

Explaining patterns arising from a complex interaction of multiple causes presents a major challenge for community ecology. At a fundamental level, an understanding of the mechanisms underlying assemblage structure and species distribution patterns cannot be achieved if those patterns are not known (Underwood *et al*., 2000). As a first step, therefore, it is necessary to undertake characterisation studies that enable the observation, identification and description of patterns in assemblage characteristics. Patterns may include, for instance, repeated grouping of particular species across sites, associations between species distributions and environmental features, or trends in compositional changes along spatial, environmental or temporal gradients. It is only after recognition of such patterns that hypotheses about their underlying causes can be formulated; these hypotheses may then be tested through further observations or manipulative experiments (Underwood *et al*., 2000; Begon *et al*., 2006).

A management perspective…

Most ecosystems on Earth are under strong human influence (Vitousek *et al*., 1997) and the oceans are no exception (Halpern *et al*., 2008; Jackson, 2008), with at least 38 distinct anthropogenic threats acting on marine ecosystems having been identified (Halpern *et al*., 2007). Such anthropogenic pressure is altering ecosystem functioning and leading to accelerated rates of biodiversity loss (Vitousek *et al*., 1997; Chapin *et al*., 2000). Establishing networks of marine protected areas (MPAs) is seen as a mechanism for conservation and sustainable management of marine biodiversity in the coastal zone, especially where tight control of human activities is necessary (Allison *et al*., 1998; Banks *et al*., 2005). In order to be effective an MPA network must be representative of the biodiversity it aims to protect. Knowledge of the distribution of biological diversity should therefore underlie the establishment of MPA networks, but this is impossible in practice given that such knowledge is often lacking (Howell *et al*., 2010). Consequently, in most cases reserve boundaries have been set on the basis of *ad hoc* evaluations with minimal ecological considerations (Fraschetti *et al*., 2005; Dauvin *et al*., 2008).

Since it is impractical to collect data on the distribution and ecology of all species within a region, focus has shifted on using benthic habitats as surrogate measures of species diversity (e.g. Ward *et al*., 1999) enabling decisions about where to locate marine reserves to be made more reliably in the absence of detailed data on the distribution of species. Two complementary approaches have been developed in this regard. The first focuses on ecologically significant or distinctive habitats. These are characterised by: (i) a high degree of 'rarity or uniqueness', such as those that contain distinct assemblages or many rare species, and/or (ii) a high degree of 'aggregation / fitness consequences', such as habitats that serve as feeding, breeding or nursery grounds for specific species, or are particularly species rich (see DFO, 2004; Derous *et al*., 2007 for criteria definitions).

The second approach focuses on ordinary habitats, where the aim is to establish a representative network of protected areas that includes examples of all the habitat types occurring in the region (Stevens, 2002). Such a network would therefore include as broad a range of species diversity as possible. However this approach can only be achieved if habitat maps at the local scale of the MPAs are available. Due to practical and economic constraints, mapping exercises often rely heavily on abiotic variables to delineate habitat borders, implicitly assuming that abiotic variables correlate with patterns of biological distributions (Stevens and Connolly, 2004). Habitats defined by abiotic factors may be too coarse-grained and require further subdivision based on specific physical factors that correlate well with species assemblages, or on ‘composition indicator’ species whose presence or abundance is indicative of a particular biological assemblage (Zacharias and Roff, 2001; Roff *et al*., 2003). Therefore, it is essential that representation is addressed via a bottom-up approach, starting by characterising the patterns in biological distributions and identifying surrogates that can be used to represent these patterns (Howell, 2010).

It is evident that the efficacy of designing MPA networks on the basis of benthic habitat maps on the assumption that habitats can act as surrogates for biodiversity is still dependent on ecological knowledge of the habitats. Characterisation of assemblages is therefore necessary to: (i) ascertain that the habitats used correlate well with, and can therefore predict, the patterns of biological distributions, and (ii) establish whether certain habitats have a higher intrinsic biological value. In addition, knowledge on the correlation between species assemblages and environmental characteristics will enable prediction of how these assemblages may change in response to environmental changes, including those associated with anthropogenic activities. Baseline information on the species assemblages characterising different habitat types is also vital for assessment of the effectiveness of MPAs, since any future shift in the species composition of assemblages cannot be evaluated without knowledge of the present state (Kipson *et al*., 2011).

1.2 Ecological aspects of sublittoral pebble-bed assemblages

Global view

In the near-shore environment, pebbles, cobbles and small boulders generally originate from regions that were previously glaciated or are highly exposed and subject to coastal erosion (Carter and Orford, 1993). Depending on the geomorphology of the coast and on the current regime present, such sediment may be washed ashore giving rise to shingle beaches, or remain in the sublittoral.

Sublittoral pebble or cobble beds are marine habitats with distinctive physical attributes (Scheibling *et al*., 2009a), but their biological characteristics have not been extensively studied. In the European Nature Information System (EUNIS) habitat classification scheme these beds fall under the “infralittoral coarse sediment” category, which also encompasses coarse sand and gravel biotopes. The pebble and cobble biotopes are generally considered to be impoverished because they tend to lack conspicuous fauna, but they may be characterised by small fauna living interstitially (Connor *et al*., 2004). However, quantitative descriptions of cobble or pebble fauna are rare, possibly because conventional sampling methods such as cores or grabs are not suitable for these habitats (Linnane *et al*., 2003).

Subtidal cobble habitats have received particular attention by researchers working on commercially exploited lobster populations, such as the American lobster *Homarus americanus* (e.g. Wahle and Incze, 1997; working in Maine, US) or European lobster *Homarus gammarus* (e.g. Linnane *et al*., 2001, 2003; working in Norway, Ireland, UK and Italy). Both studies by Linnane *et al*. (2001, 2003) recorded the abundance of other macrofauna besides the European lobster itself, but their discussions are limited to comments on the ability of cobble beds to act as nursery habitat for decapod crustaceans. This aspect of cobble-bed ecology was investigated more fully by Robinson and Tully (2000a) who compared the recruitment and community structure of decapods in subtidal habitats having different physical structure. Their results suggest that cobble habitats are in fact important as nursery grounds by providing shelter, and that habitat characteristics such as physical complexity contribute to spatial differences in the decapod assemblages.

Robinson and Tully (2000b) studied the seasonal variation in decapod assemblages associated with subtidal cobble substrata and found that community structure differed between the settlement season and overwintering periods. Seasonal differences were also noted in the case of macrophytes (Lieberman *et al*., 1979, 1984; Davis and Wilce, 1987a, 1987b) and sessile fauna (Osman, 1977). These studies have demonstrated the unstable nature of cobble habitats, showing an inverse relationship between cobble size and its frequency of overturn, and hence disturbance. For instance, Osman (1977) attributed the differences in sessile biota between differently sized cobbles/boulders to the length of time since the last overturn, that is, the time interval over which colonisation could proceed, while Lieberman *et al*. (1979) and Davis and Wilce (1987a) suggested that in the case of algae, the differences were a result of the increased removal of erect macrophytes from the smaller and more frequently disturbed cobbles.

Although physical factors, especially disturbance, appear to be important in structuring the biotic communities of sublittoral cobble or pebble beds, biological interactions may also play a role. For instance, Scheibling and Raymond (1990) recorded an increase in biomass of erect algae and in the abundance of herbivorous molluscs following mass mortalities of sea urchins, although crustose coralline algae remained the dominant algae, while other macrofauna were not affected. Subsequent work highlighted the role played by herbivorous molluscs in controlling early succession and community organisation of algal species (Scheibling *et al*., 2009b). The size of the erect algae can itself influence the degree of physical disturbance: cobbles with large fleshy algae that reach a critical mass (relative to that of the anchoring cobble) are more easily displaced than similar-sized cobbles lacking macroalgae; thus algal-mediated displacement of cobbles can be an important form of physical disturbance (Scheibling *et al*., 2009a).

Overall, these studies indicate that cobble or pebble substrata are dynamic environments, where disturbance plays an important role in structuring the community, particularly with respect to sessile species. On the other hand, very little information on its effects on mobile biota is available, while other ecological aspects such as the variation in macrofaunal composition in relation to the physical structure of the substratum have not been extensively investigated.

Mediterranean infralittoral pebble beds

In Mediterranean wave-dominated rocky shores, coarse sediments deposit at the enclave between the rocky platforms extending from the shore and the sedimentary bottoms found in deeper waters (Riedl, 1971). Accumulations of pebbles and cobbles typically occur in wave-exposed rocky coves down to a depth of a few decimetres (Pérès and Picard, 1964; Ros *et al*., 1985). This shallow-water pebble substratum supports a distinct biotic assemblage, which was originally designated by Pérès and Picard (1964) as the biocoenosis of infralittoral pebbles, based on their work in the south of France.

Pérès (1967) described this biocoenosis as follows (currently valid nomenclature of species is included in square brackets):

> “Small strands with infralittoral pebbles are very numerous in the indentations of rocky coasts. The most typical is the biocoenosis when the pebbles are rather small. The characteristic species here are the two amphipods *Melita hergensis* and *Allorchestes aquilinus* [*Parhyale aquilina*], which feed on organic detritus, and the fish *Gouania wildenowii* [*G. willdenowi*], predator on these amphipods. One finds also a characteristic crab, *Xantho poressa*, and several turbellerians and nemertines. After long periods of calm a thin film of diatoms is found on the pebbles. When the pebbles are larger this typical biocoenosis is often impoverished, but mixed with tolerant species coming from neighbouring rocks such as the sea-star *Asterina gibbosa*, the small anomuran *Porcellana bluteli* [*Pisidia bluteli*], the gobiescoid fish *Lepadogaster gouani* [*L. lepadogaster*], and so on. During stormy weather all the species escape from the moving pebbles to the lower parts of the boulders generally mixed with the pebbles, or to deeper bottoms, to return as soon as the sea becomes calm.”

According to Bellan-Santini (1985) this biocoenosis is impoverished, but she concedes that this habitat has not really been studied. The situation has not changed much since then, to the extent that almost no information about this biocoenosis is provided in the synthetic accounts of Ros *et al*. (1985) and Bellan-Santini *et al*. (1994), or even in recent interpretive manuals for the identification of the different biocoenoses (e.g. Bellan-Santini *et al*., 2002; Relini and Giaccone, 2009). Indeed, the descriptions by Pérès and Picard (1964; in French) and Pérès (1967; see above) appear to be the most complete available to date.

Very little information beyond that included in biocoenotic descriptions is available, and virtually no studies on this assemblage appear to have been published in the mainstream literature. However, Mediterranean catalogues of marine amphipods and molluscs (e.g. Ruffo, 1982, 1989, 1993, 1998; Cachia *et al*., 1991, 1996, 2001), list “under stones” as the microhabitat of several species, indicating that infralittoral pebble beds may in fact be more species diverse than has been suggested.

In addition, it seems likely that the presently available descriptions do not cover the entire breadth of this habitat. For instance, in their search for the early benthic phase of the European lobster, Linnane *et al*. (2001) recorded a total of 20 decapod species from a sampling site at Elba Island, Italy, where the substratum consisted of cobbles on a sand and shingle bottom at depths of 9–13 m. Similarly, Evans (2007) found 69 species of molluscs among accumulations of cobbles and pebbles occurring at depths of 5–12 m in Marsamxett, Malta. Thus, neither site fits into the physical or biotic descriptions of the Mediterranean infralittoral pebble habitat given by Pérès and Picard (1964) and subsequent authors.

1.3 The Maltese Islands

Physical Geography

The Maltese Islands are a group of small, low-lying islands aligned northwest-southeast and situated in the Sicilian Channel at the centre of the Mediterranean, approximately 96 km from Sicily and 290 km from North Africa. The islands are tilted towards the north-east, such that cliffs dominate the south-western coasts whereas gently sloping shores are found on the north-eastern shores (Schembri, 1997). The gentle gradient on the north-eastern shores extends seaward, with the depth in this area remaining less than 50 m up to 1–2 km away from the coast, while further afar the sea between Malta and Sicily is mostly less than 90 m deep and reaches a maximum depth of only ca. 200 m. In contrast, off the south-western coast the sea reaches a depth of 50 m within 200–500 m away from the shore, and the sea between Malta and North Africa can reach depths exceeding 1000 m in places (Morelli *et al*., 1975).

The Maltese archipelago consists of three inhabited islands (Malta, Gozo, and Comino) together with a number of small satellite islands and rocks, occupying a total area of 316 km^2. The 271 km of coastline are predominantly rocky (90.5%), with mobile sediments accounting for only 2.4% of the shore; the rest of the shoreline consists of artificial structures (Mallia *et al.*, 2002; Schembri *et al.*, 2005). The rocky shoreline extending sublittorally is typically replaced by sedimentary bottoms in moderately deep waters, and pebbles and cobbles may occasionally accumulate at the border between the hard and soft substrata, particularly in small coves and inlets (Borg and Schembri, 2002).

The climate of the Maltese Islands is strongly biseasonal, and is characterised by mild, wet winters and hot, dry, sunny summers. Around 85% of the total annual rainfall occurs between October and March, but even this wet season may include long dry periods, with short-duration heavy downpours accounting for most of the annual rainfall. No rivers or streams are present, so terrestrial runoff into the sea is ephemeral, occurring only during periods of heavy rainfall (Schembri, 1997; Galdies, 2011).

The marine climate is also biseasonal. In winter, the strong winds and waves associated with stormy conditions, and the increase in density of surface water on cooling, result in vertical mixing of the water column (Flos, 1985). Thus, a homogeneous water column with temperatures of around 16°C in the 0–50 m depth range occurs in winter (Drago *et al.*, 2010). In contrast, the relatively calm weather conditions that prevail during spring and summer (Galdies, 2011) together with warming of surface waters due to the higher air temperatures, lead to the formation of a summer thermocline. Sea surface temperatures reach a peak of around 27°C in August, compared to temperatures at depths greater than 30 m which remain below

18°C (Woods, 1968; Drago *et al*., 2010). The shape and steepness of the temperature-depth profile can vary depending on weather conditions (Flos, 1985) but the maximum thermal gradient normally lies at the 20–25 m depth range (Woods, 1968).

The presence or absence of a shallow-water thermocline defines seasonality in Maltese coastal waters: the summer season is characterised by calm hydrodynamic conditions that enable the thermocline to form and persist, whereas turbulent hydrodynamic regimes that cause the thermocline to break down prevail during the winter season; wave energy is therefore higher during winter (Scott Wilson, 2003). Given that Maltese shores are microtidal (maximum tidal range: 20 cm; Drago, 2009), wave energy is the main source of physical disturbance to shallow-water benthic assemblages. Thus, the hydrodynamic conditions during winter storms can be an important agent of disturbance, potentially leading to seasonal changes in composition and structure of benthic assemblages, particularly those associated with mobile substrata such as pebble beds.

Marine Biogeography

The Mediterranean Sea is a biodiversity hotspot (Lejeunse *et al*., 2010), hosting around 6-7% of the global number of marine species (excluding microbes), in spite of occupying only 0.82% of the ocean surface (Bianchi and Morri, 2000; Coll *et al*., 2010). Recent estimates indicate that approximately 17,000 marine species occur in the Mediterranean (of which ca 26% are microbes), but knowledge on certain groups is very limited (Coll *et al*., 2010). Species richness declines along a northwest-to-southeast direction; thus the highest diversity is found in the western Mediterranean, followed by the central Mediterranean, Adriatic, and Aegean seas (Koukouras *et al*., 2001; Coll *et al*., 2010).

The high diversity is attributed to the complex geological history of the Mediterranean (Maldonado, 1985; Bianchi *et al*., 2012) and to the present-day variation in climatic and hydrologic conditions occurring in different regions of this sea, leading to the occurrence of both temperate and subtropical biota, as well as a high level of endemism (c. 20%) (Bianchi and Morri, 2000; Coll *et al*., 2010; Bianchi *et al*., 2012). One species (the trochid gastropod *Gibbula nivosa*) is actually endemic to the Maltese Islands, making it a rare case of marine point-endemism (Evans *et al*., 2011). No estimates of the total species richness specifically for Maltese marine biota have been published, but checklists of Italian marine species (Relini, 2008, 2010) include a sub-zone encompassing the south-eastern tip of Sicily, the Pelagian Islands and the Maltese archipelago, with a total of 4,009 species. However, this is almost certainly an underestimate since certain groups are poorly represented due to inadequate knowledge (including Archaea, Bacteria, Protista and Fungi, phytoplankton as well as numerous animal groups).

Differences in the distribution of biota within the Mediterranean have led to the recognition of distinct biogeographic sectors. One of the major boundaries is that between the western and eastern regions of the Mediterranean (Bianchi, 2007), and several attempts at defining the position of this boundary have been made. For instance, Pérès and Picard (1964) placed this boundary in the Ionian Sea, including the whole Sicily and Malta in the western Mediterranean biogeographic sector, whereas according to Giaccone and Sortino (1974) the position of the boundary between the western and eastern biogeographic sectors lies in the middle of the Straits of Sicily, such that Malta is included in the eastern bioregion.

Bianchi and Morri (2000) traced the boundary of ten biogeographical zones in the Mediterranean (Figure 2.1). In this scheme, the Maltese Islands not only mark the junction between the western and eastern zones but also that between the northern and southern bioregions. The exact positions of these boundaries vary among different authors because they depend on which groups of species are used to define the biogeographic sectors. Additionally, although the core zones of the different regions can be easily identified, tracing their boundaries on a map can be difficult (Bianchi, 2007). Nonetheless, it is clear that the Maltese Islands are located close to some of the main biogeographical boundaries in the Mediterranean, and thus Maltese marine biota has affinities to practically all the biogeographic regions of this sea.

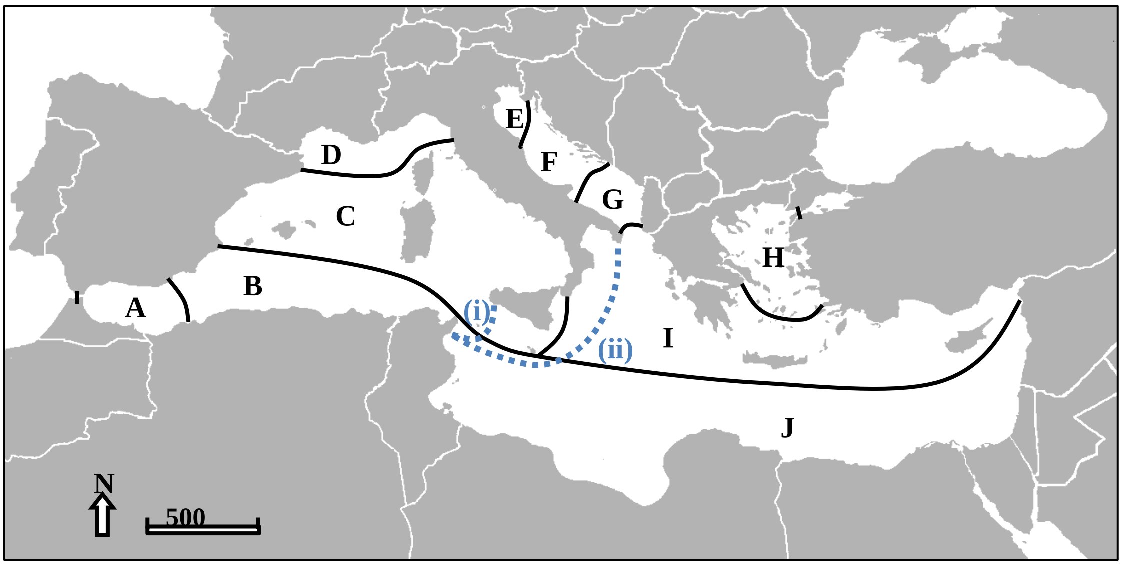

Figure 1.1 Map of the Mediterranean Sea indicating the boundaries of the main biogeographic sectors (A – J) according to Bianchi and Morri (2000) [solid black lines] and the position of the boundary between the western and eastern bioregions according to (i) Giaccone and Sortino (1974) and (ii) Pérès and Picard (1964) [dotted blue lines].

1.4 The present study

Aims and objectives

Given that only limited information is available on the diversity of the pebble-bed biotic assemblage, on the spatial and temporal variations in assemblage structure, or on interactions between physical factors and the biotic components, there is scope for further studies to address the gaps in knowledge on the physical and biological characteristics of these assemblages, especially in the Mediterranean region where no such studies have been carried out. Since the Maltese Islands are located at the biogeographic boundary between the western and eastern Mediterranean bioregions (Bianchi, 2007), the Maltese marine biota has elements from practically all the biogeographic regions of the Mediterranean and thus the Maltese Islands are ideally situated for characterisation studies on Mediterranean habitats.

Therefore, the overall aim of this study is to characterise the infralittoral pebble-bed assemblages found in the Maltese Islands in order to provide insights into the ecological dynamics of this assemblage. The specific objectives of the study are:

- to locate different types of infralittoral pebble habitats and map their distribution around the Maltese Islands;
- to characterise the environmental characteristics of these pebble beds and quantify the spatial variation in abiotic factors;
- to identify the biotic components of the pebble-bed assemblages, determine whether distinct assemblage types occur, and identify environmental features that are correlated with spatial variation in assemblage structure;

- to quantify the extent of temporal variation in infralittoral pebble-bed assemblage structure and composition, as related to seasonal disturbance;
- to examine the patterns in species diversity of the pebble-bed assemblage within a specific region in order to make inferences on the underlying mechanisms determining these patterns.

Thesis outline

Each of the chapters presented in this thesis has been written as a separate piece of research, and can therefore be read in isolation without the need for cross-reference. Consequently, some repetition may be present in the different chapters, especially with regards to the field and laboratory methodologies. The individual chapters address the above objectives as follows:

- Chapter 2 focuses mainly on the physical characterisation aspects, including the identification of: (i) different types of pebble habitats based on their abiotic features, and of (ii) the subset of environmental features that best explain variation in the biotic assemblage. The outcomes provide insights into the relative role of these physical variables in influencing the biotic structure of pebble-bed assemblages.
- Chapter 3 deals with the biological characterisation aspects, focusing on: (i) the classification of assemblages into distinct types according to their biotic components, and (ii) evaluation of the variation in assemblage structure among these different types. This leads directly to the development of an ecologically

meaningful habitat classification scheme for pebble-bed habitats, which can be used in mapping surveys.

- Chapter 4 investigates the biotic changes occurring in shallow-water (<2 m depth) infralittoral pebble beds as a consequence of seasonal disturbance, based on biannual sampling over a two-year period. Although the role of disturbance was already the subject of previous studies on cobble habitats, this is the first study which focuses on the motile fauna rather than sessile biota.

- Chapter 5 details the spatial patterns in species diversity of infralittoral pebble beds found in the Marsamxett Harbour area, which includes those pebble-bed sites having the highest richness among all those investigated (as described in Chapter 3). Diversity is partitioned into inventory (alpha) and differentiation (beta) components to determine their relative contribution to overall regional (gamma) diversity. The underlying mechanisms for among-site variation in diversity are also investigated.

Although each chapter is presented as an independent study, it is intended that in combination the chapters provide a better understanding of the ecological dynamics of infralittoral pebble-bed assemblages. Accordingly, a general discussion of the overall findings of the present work, integrating the outcomes of the individual studies and assessing their implications for conservation management of pebble-bed habitats, is provided in Chapter 6.

Chapter 2:

Physical characterisation of infralittoral pebble beds in the Maltese Islands

Chapter 2

Physical characterisation of infralittoral pebble beds in the Maltese Islands

Abstract

The Mediterranean biocoenosis of infralittoral pebbles has been poorly studied and very little is known about the physical characteristics of such habitats, or their relationship with the associated biotic assemblages. This study characterised nineteen pebble beds in the Maltese Islands in terms of their location, area, structure, particle size and shape, and physico-chemical attributes of the water; correlations between these parameters and assemblage structure were also identified. Pebble beds were classified into three different bed types based on the measured physical attributes. These included pebble beds occurring: (i) in very shallow waters (<2 m depth), (ii) within creeks or in inter-matte regions of seagrass meadows, and (iii) in harbour environments. Variation in the pebble-bed biotic assemblages was associated with differences in fine particle content (a proxy for hydrodynamism), water temperature, pebble-bed thickness, and size of the habitat patches, suggesting that environmental control is important in determining assemblage structure. This information will enable better assessment of the potential impacts of anthropogenic disturbance on pebble-bed assemblages.

2.1 Introduction

One of the major goals of ecology is to understand the mechanisms determining species distributions and the structure of communities. Environmental features play a major role in determining community composition since the abiotic environment can exert a negative forcing on the performance of organisms (Scrosati *et al*., 2011). Species are therefore more likely to occur in habitats that provide optimal conditions for their survival, leading to co-occurrence of species with similar habitat requirements and segregation of species having affinities for non-overlapping habitats (Belyea and Lancaster, 1999). Habitat features act as filters selecting for, or against, particular functional traits, and hence determining the probability that a given species will persist within a community (Keddy, 1992; Poff, 1997).

Biotic interactions such as interspecific competition for resources can also serve as a potential filter on local community composition (e.g. Belyea and Lancaster, 1999, and references therein). However, disentangling the contributions of competitive interactions or individual species requirements to species co-occurrence patterns is not straightforward and many different hypotheses can be invoked to account for the same non-random patterns (Gotelli and McCabe, 2002). Furthermore, different processes may operate simultaneously, such that local community composition depends on a complex interplay between both abiotic and biotic factors (Menge and Olson, 1990; Hilborn and Stearns, 1992). The prevailing abiotic environment may also alter the interspecific interactions (Menge and Olson, 1990, Menge *et al.,* 2002) which may even switch from negative (e.g. competition) to positive (e.g. facilitation) interactions with increasing levels of environmental stress (Bertness *et al*., 1999).

Habitat features are thus major determinants of community structure, whether by serving as environmental constraints directly limiting which species form part of the community, or through influencing the biotic interactions between these species. As a starting point, understanding the relationship between organisms and their habitat requires quantifying relevant habitat parameters that are crucial in determining the community structure (Wilding *et al*., 2007). This may seem trivial for habitats that have already been the focus of extensive studies, especially where these included studies aimed at identifying environmental correlates of assemblages, or specifically testing the effect of changes in particular habitat features on community organisation. On the other hand, not all habitats have been studied in detail, and sublittoral pebble beds are a case in point.

In the Mediterranean sublittoral pebble beds are thought to give rise to a unique assemblage known as the ‘biocoenosis of infralittoral pebbles’ (UNEP-MAP-RAC/SPA, 2006a). Pérès and Picard (1964) originally described this habitat as occurring in creeks along rocky coasts, down to a depth of just a few decimetres, and being characterised by pebbles that rarely exceed a few centimetres in diameter. In addition, Pérès (1967) notes that infralittoral pebbles generally occur in exposed locations and are gradually substituted by coarse sands and fine gravels when wave exposure is experimentally reduced (Picard, 1962). Subsequent publications on Mediterranean habitats (Bellan-Santini, 1985; Ros *et al*., 1985; Bellan-Santini *et al*., 1994; Bellan-Santini *et al*., 2002; Relini and Giaccone, 2009) provide no additional details on the physical characteristics of infralittoral pebble beds.

Therefore, only limited information on physical features of the Mediterranean pebble-bed habitat is available. Parameters such as the structure and thickness of the pebble layer or even the area of the pebble patches have not been quantified to date, in spite of their potential biological relevance. Furthermore, no assessment of spatial variation in any of these parameters appears to have been carried out and it is likely that the presently available descriptions do not cover the entire breadth of this habitat. For instance, in their search for the early benthic phase of the European lobster *Homarus gammarus*, Linnane *et al*. (2001) included a sampling site at Elba Island, Italy, where the substratum consisted of cobbles on a sand and shingle bottom at depths of 9–13 m. Similarly, Evans *et al*. (2010, 2011) listed accumulations of cobble and pebbles at depths of 5–12 m in Marsamxett Harbour, Malta as the preferred habitat of the Maltese top-shell *Gibbula nivosa*. A total of 20 decapod species were recorded at Elba Island (Linnane *et al*., 2001), while 69 species of molluscs were collected from Marsamxett Harbour (Evans, 2007), suggesting that these pebble beds are not as impoverished as generally thought (e.g. Bellan-Santini, 1985). Thus neither site fits into the physical or biotic descriptions of the Mediterranean infralittoral pebble habitat given by Pérès and Picard (1964) and subsequent authors.

The dearth of information on sublittoral pebble and cobble habitats is not unique to the Mediterranean region. In the 2012 European Nature Information System (EUNIS) habitat classification scheme infralittoral pebble beds fall under habitat code A5.13 (Infralittoral coarse sediment). This includes five cobble/pebble/shingle habitat types in addition to the Mediterranean one, with a very brief description provided for only two of these (Habitat A5.131 – “Sparse fauna on highly mobile sublittoral shingle”, and Habitat A5.132 – “*Halcampa chrysanthellum* and *Edwardsia timida* on sublittoral clean stone gravel”).

On a global scale, very few studies on temperate sublittoral pebble or cobble habitats have been published in the mainstream literature, and these are limited to just six locations: Nonamesset Island, Massachusetts, USA (Osman, 1977); Plum Cove, Massachusetts, USA (Davis and Wilce, 1987a, 1987b); Eagle Head, Nova Scotia, Canada (Scheibling and Raymond, 1990; Scheibling *et al*., 2009a, 2009b); Saltees Sound, Ireland (Robinson and Tully, 2000a, 2000b); Galway Bay, Ireland (Linnane *et al*., 2003); and Vernon Bank, Ghana (Lieberman *et al*., 1979, 1984). Most of these have focused on the effect of disturbance on macrophytes or sessile biota; only Robinson and Tully (2000a) have investigated the spatial variability in community structure (of decapods) in relation to substratum characteristics, but they also included areas that consisted predominantly of coarse sand or bedrock amongst their five sites.

Within this context, the present study was carried out to quantify the range and spatial variability in physical characteristics of infralittoral pebble beds in the Maltese Islands, thus providing detailed habitat data for the Mediterranean biocoenosis of infralittoral pebbles for the first time. Malta was an appropriate starting point for studying this habitat in the Mediterranean given its geographical location at the centre of this sea, and the fact that pebble beds that do not fit in within the current benthic habitat classification schemes for this region had already been observed there (Evans, 2007). Such baseline data can serve for comparative purposes if pebble habitats are studied elsewhere in the Mediterranean.

A second objective of this study is to identify correlations between the physical parameters and biotic components of the habitat. Recognition of such patterns is a necessary precursor for the formulation of hypotheses on the relationship between the physical nature of the pebble habitat and the species assemblage it supports

(Underwood *et al*., 2000). Knowledge on the correlation between species assemblages and environmental characteristics is also vital for conservation efforts, since it enables prediction of how these assemblages may change in response to environmental changes, including those associated with anthropogenic activities. Characterisation of the biological components of these habitats is treated in Chapter 3.

2.2 Material and methods

Study sites

A preliminary survey was carried out by snorkelling along the low-lying coasts of the Maltese Islands in order to map the presence of infralittoral pebble beds. Surveying was carried out during January–March 2011. Small accumulations of pebbles were observed in numerous locations, mostly along the eastern shores (cliffs dominate the western coast). These often occurred as enclaves within other habitats and had minimal coverage, but more extensive beds also occurred in some locations.

Suitable study sites were selected according to the following criteria: (i) the area consisted predominantly of pebbles (stones having a diameter of 2–10 cm following the UNEP-MAP-RAC/SPA [2006a] criteria), which lacked a covering of erect macrophytes; (ii) an area of at least 25 m^2 of pebble habitat was present at a depth of more than 0.5 m below chart datum. Areas with cobbles or small boulders that had a permanent cover of erect algae were excluded because they are considered to belong to a different biocoenosis – that of infralittoral algae (e.g. Pérès and Picard, 1964; Bellan-Santini *et al*., 1994).

Fifteen locations were selected according to the above criteria. Since most of these locations had a small coverage (typically <50 m^2), it was not possible to nest more than one site within a single location without leading to pseudoreplication (Hurlbert, 1984); on the other hand the relatively large beds (>1000 m^2) present at Tigné and Ta' Xbiex and presence of two separate beds at Manoel Island and Wied ix-Xoqqa permitted use of two sites at each of these four locations. Therefore a total of nineteen sites were surveyed in the present study (see Table 2.1 and Figure 2.1).

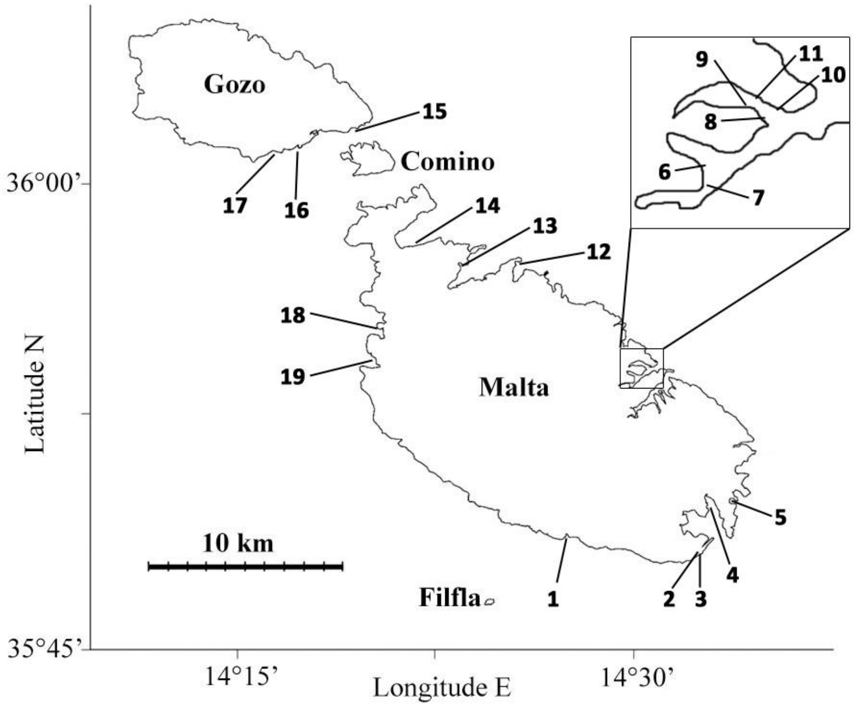

Figure 2.1 Map of the Maltese Islands indicating the position of the nineteen sampling sites used in the present study. Inset shows enlarged view of Marsamxett Harbour. Site numbers correspond to those listed in Table 2.1.

Based on their physical setting, the studied pebble beds could be classified into four categories. These include pebble beds occurring (i) in very shallow waters (<2 m) within small rocky coves, (ii) in slightly deeper regions within creeks (with only ephemeral freshwater input), (iii) within inter-matte regions of reticulate *Posidonia oceanica* beds, or (iv) in harbour environments; these are hereafter referred to as ‘shallow’, ‘creek’, ‘seagrass’ and ‘harbour’ pebble-bed types respectively. Classification of the nineteen sites into these four pebble-bed types is shown in Table 2.1.

Table 2.1 Pebble-bed sites used in the present study, together with their respective geographical coordinates (WGS84 datum) and initial categorisation into different bed types based on seascape features in the field.

Site		**Coordinates (Latitude/Longitude)**		**Bed type**
1.	Wied iz-Zurrieq	35° 49.238'N	14° 27.119'E	Creek
2.	Wied ix-Xoqqa A	35° 48.684'N	14° 32.327'E	Shallow
3.	Wied ix-Xoqqa B	35° 48.623'N	14° 32.361'E	Creek
4.	Marsaxlokk	35° 50.030'N	14° 32.710'E	Shallow
5.	Hofra z-Zghira	35° 50.281'N	14° 33.597'E	Shallow
6.	Ta’ Xbiex A	35° 53.987'N	14° 30.068'E	Harbour
7.	Ta’ Xbiex B	35° 53.904'N	14° 30.054'E	Harbour
8.	Manoel Island A	35° 54.253'N	14° 30.462'E	Harbour
9.	Manoel Island B	35° 54.319'N	14° 30.382'E	Harbour
10.	Tigné A	35° 54.368'N	14° 30.593'E	Harbour
11.	Tigné B	35° 54.410'N	14° 30.467'E	Harbour
12.	Qawra	35° 57.559'N	14° 25.571'E	Shallow
13.	Mistra	35° 57.462'N	14° 23.444'E	Seagrass
14.	Tunnara	35° 58.010'N	14° 21.441'E	Shallow
15.	Hondoq ir-Rummien	36° 01.672'N	14° 19.509'E	Seagrass
16.	Xatt l-Ahmar	36° 01.153'N	14° 17.292'E	Creek
17.	Mgarr ix-Xini	36° 01.177'N	14° 16.323'E	Creek
18.	Gnejna	35° 55.423'N	14° 20.546'E	Seagrass
19.	Fomm ir-Rih	35° 54.711'N	14° 20.131'E	Seagrass

Sediment sampling and analysis

At each site, divers measured the water depth using a digital depth gauge and estimated the dimensions of the pebble beds with fibreglass tape measures. Four replicate samples of the substratum were subsequently collected from each of the 19 sites; their position was selected at random, but no samples were collected towards the edges of the pebble beds to avoid edge effects (e.g. Todd and Turner, 1986). Sampling was carried out using a 0.1 m^2 circular sampler (modified from Borg *et al*., 2002) which was pushed into the substratum to delineate the sampling area and prevent escape of motile invertebrates. Cobbles and pebbles were hand-collected and transferred to a 0.5 mm mesh bag, while a small fine-mesh (1 mm mesh size) hand net was used to scoop the basal layer of finer granules. A suction sampler was simultaneously employed to reduce the risk of missing highly-motile organisms, thus ensuring quantitative samples were collected. The thickness of the pebble layer was then estimated by measuring the thickness of the exposed section in comparison to the adjacent undisturbed region, using a 30 cm ruler. Finally, a small core sample (area: 0.0016 m^2) of the granule layer and underlying fine sediments up to a depth of 5 cm was collected for grain-size analysis. All fieldwork was carried out between July and September 2011.

Samples were transported to the laboratory and preserved in 10% formaldehyde in seawater. The samples were subsequently rinsed and sorted, separating the macrofauna (retained by a 0.5 mm sieve; e.g. Castelli *et al.*, 2003) into major taxonomic groups, while retaining the sediment for granulometric analysis. The biota were identified to the lowest taxon possible and enumerated to construct a species-abundance matrix.

For particle-size analysis, all the pebbles greater than 8 mm were manually sorted into size classes at half-phi intervals on the Udden-Wentworth scale (i.e. eight size classes in the 8–128 mm range) using a gravel sizing template (Hydro Scientific Ltd.). Smaller particles were first treated with sodium hexametaphosphate and wet-sieved for the determination of silt content, then dried and separated into size classes at phi-intervals using a series of nested test-sieves (0.063–8 mm) shaken on a mechanical sieve-shaker for 20 minutes at moderate amplitude (see Bale and Kenny, 2005). Since the finer sediment was collected using smaller corers, the masses of the particle-size fractions less than 8 mm were scaled up using the ratio of the two corers as the scaling factor.

Grain-size data were analysed via the method of moments to obtain the geometric mean particle size using the GRADISTAT program (Blott and Pye, 2001). Since bimodal distributions were common, the mean particle size for the gravel fraction (particles >2 mm) was calculated independently of any finer particles, which were then quantified separately in terms of the percentage sand and silt content. Note that since sorting of particles into size classes was carried out using sieving techniques, mean grain size was measured in terms of the sieve diameter, which reflects the cross-sectional diameter of the particles not their maximum length.

Other sediment characteristics of potential relevance to the biotic structure that required measurements of individual pebbles were carried out on subsample of pebbles. For each of the 76 samples, ten pebbles were randomly selected from each size fraction (>8 mm), and the mean value of the measured parameters estimated using a weighted average. Following the recommendations by Bergey and Getty (2006), surface area was estimated from the maximum dimensions of the particles along three orthogonal axes (denoted L, W and H) according to the equation *Surface Area* = $1.15*(LW+LH+WH)$ (see Graham *et al*., 1988).

Stone resistance to rotation along its major axis was calculated as the moment of inertia of an ellipsoid ($I = m*(b^2+c^2)/5$; where m is the mass, and b and c are the lengths of the two minor semi-axes). Particle shape was characterised by its: (i) form, estimated in terms of deviation from equancy with respect to elongation (W/L ratio) and flatness (H/W ratio; see Blott and Pye, 2008); (ii) roundness, using the visual comparator proposed by Blott and Pye (2008); and (iii) sphericity, calculated as the square-root of the ratio of the largest inscribed circle diameter to the smallest circumscribed circle diameter (Riley, 1941) averaged across the three dimensions of the pebbles. Percentage cover of encrusting and filamentous algae was also recorded.

Physico-chemical characterisation of the water

Since some of the sites were located in locations with potential influence by freshwater run-off, physico-chemical characterisation of the water at the surface of the pebble beds was also undertaken. This included monthly measures of temperature, salinity, dissolved oxygen and turbidity, carried out using an *in situ* meter (YSI 6820 multiparameter sonde connected to a YSI 650MDS meter) deployed just above the seafloor. Two replicate seawater samples were also collected on a monthly basis from just above the pebble habitat using a Van Dorn water sampler. These samples were collected in polycarbonate bottles and transported to the laboratory in cooler boxes maintained at 4°C, where they were subsequently stored at -20°C until analysis. Chemical analyses for nitrate and phosphate levels were carried out using an automated continuous flow analyser (Skalar SAN++ coupled with an SA1000 autosampler). Annual average concentrations for these parameters were subsequently calculated following standard practises in water quality monitoring (EC, 2009).

Statistical analyses

Spatial variation in physical attributes was analysed using multivariate techniques: Principal Components Analysis (PCA) was used to visualise the ordination pattern of sites, while CLUSTER analysis was used to group sites with similar physical characteristics. Similarity Profile (SIMPROF; Clarke *et al*., 2008) permutation tests (at 1% significance level) were carried out at every node of the completed dendrogram to determine whether there was significant sub-group structure within clusters. Distance-based multiple regression and redundancy analyses (DistLM and db-RDA; McArdle and Anderson, 2001) were then used to identify the physical variables correlated with variation in the biotic assemblage (based on species abundance data).

Although the DistLM/db-RDA routines do not require the assumption of multivariate normality, predictor variables should not be heavily-skewed or contain extreme outliers (Anderson *et al*., 2008). PCA also performs better if the variables have an approximately normal distribution (Legendre and Legendre, 1998). Therefore, prior to these analyses, the physical variables were individually examined via construction of frequency-distribution histograms and Shapiro-Wilk normality tests (Shapiro and Wilk, 1965) and, where necessary, Box-Cox normalising transformations were applied (see Sokal and Rohlf, 1995). Since abiotic variables were measured on different measurement scales they were also standardised to unit variance to bring them to a common scale, enabling their use in multivariate analysis (Legendre and Legendre, 1998).

Euclidean distance is an appropriate measure of among-sample dissimilarity when using environmental variables (e.g. Clarke, 1993) and was therefore used to construct the resemblance matrix for the CLUSTER analysis; it is also the distance measure preserved among objects in PCA (Legendre and Legendre, 1998). DistLM/db-RDA model the relationship between the biological assemblages and known variation in the environment, but the analyses depend on the choice of resemblance measure for the biotic data. Two measures of dissimilarity were used here: χ^2 distance and Bray-Curtis similarity. The Bray-Curtis coefficient is often the preferred choice for analysis of species-abundance data (Legendre and Legendre, 1998; Clarke, 1993) but could not be used in direct gradient analysis prior to the development of db-RDA techniques by Legendre and Anderson (1999). On the other hand, the χ^2 distance metric is widely used for modelling species-environment relationships (for instance in Correspondence Analysis and its derivatives) since it provides a good approximation for species with unimodal distributions along a single environmental gradient (ter Braak and Verdonschot, 1995; Lepš and Šmilauer, 2003).

Since Bray-Curtis similarity tends to be unduly dominated by counts for highly abundant species, while rare species may have an unduly large influence on the analysis based on χ^2 distance, the species abundance data were square-root transformed to down-weight the importance of highly abundant species (Clarke and Green, 1988), and truncated to remove the rarer species, retaining only those making up more than 1% of the total abundance in at least one of the samples. DistLM was carried out using the transformed environmental variables after checking for multi-collinearity (no variables were removed since Pearson's r was < 0.9 in all cases). Forward sequential fitting was used to select those variables with the highest explanatory power according to the adjusted-R^2 selection criterion; db-RDA plots were subsequently produced to allow the visualization of the sites' ordination according to the multivariate regression models previously generated via DistLM.

2.3 Results

A summary of the various physical parameters quantified for each of the four pebble-bed types is provided in Table 2.2. Shallow beds rarely exceeded a depth of 1 m, whereas seagrass, creek and harbour bed types were generally found within the 5–10 m depth range. Extensive beds (>500 m^2) were only present in the latter category, with patches of pebbles in the other categories rarely exceeding 50 m^2. In all cases, the pebble bed had a layered structure, with an upper stratum of pebbles and a lower stratum of granules and sand that became progressively finer. In the deeper sites, particularly within the harbours, a basal silty layer was also present (Figure 2.2).

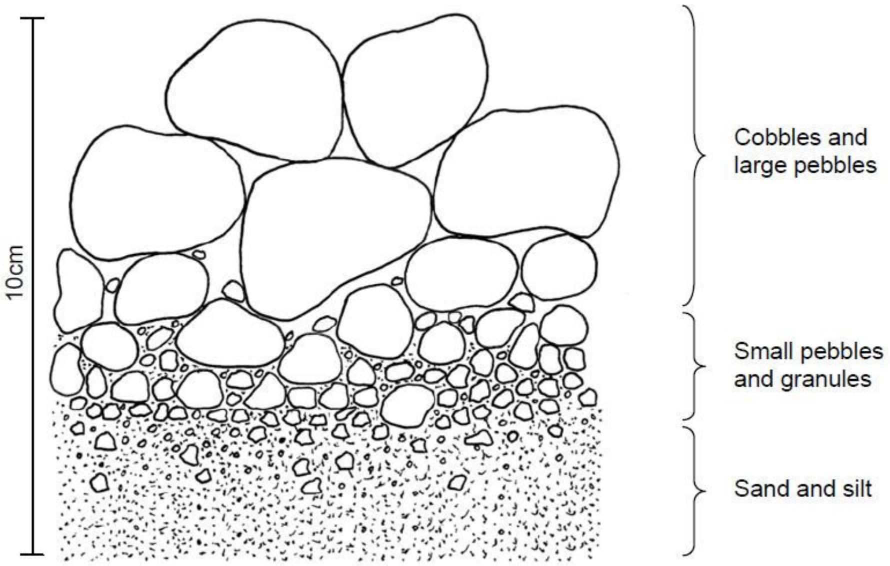

Figure 2.2 Pebble-bed profile showing vertical stratification, with an upper layer of pebbles and a lower layer of granules and sand; a basal layer of silty sand was typically present in harbour environments.

Table 2.2 Mean (± SD) values of the physical parameters measured in the present study for each of the four pebble-bed types (see Table 2.1), together with the overall mean.

	Overall	**Shallow**	**Seagrass**	**Creek**	**Harbour**
Depth (m)	5.0 ± 3.4	0.9 ± 0.3	6.5 ± 4.4	5.5 ± 2.1	7.1 ± 1.9
Area (m^2)	806 ± 1422	45 ± 31	49 ± 35	124 ± 120	2400 ± 1677
Thickness (cm)	5.28 ± 1.65	6.80 ± 1.47	5.31 ± 1.57	3.56 ± 1.48	5.13 ± 0.72
Mean pebble size (mm)	30.7 ± 15.0	39.6 ± 21.5	33.9 ± 16.9	26.1 ± 12.3	24.3 ± 4.9
Sand & Silt content (%)	18.9 ± 12.8	5.8 ± 4.0	16.3 ± 5.3	14.8 ± 6.0	34.3 ± 8.2
Mean moment of inertia (g cm^2)	198 ± 419	564 ± 729	125 ± 165	82 ± 82	19.6 ± 5.4
Total surface area (m^2)	0.54 ± 0.20	0.76 ± 0.19	0.51 ± 0.07	0.47 ± 0.25	0.43 ± 0.05
Mean Elongation	0.74 ± 0.02	0.74 ± 0.01	0.74 ± 0.02	0.74 ± 0.02	0.74 ± 0.02
Mean Flatness	0.66 ± 0.04	0.63 ± 0.05	0.67 ± 0.04	0.67 ± 0.01	0.66 ± 0.05
Mean Roundness	0.69 ± 0.05	0.70 ± 0.04	0.67 ± 0.06	0.64 ± 0.02	0.72 ± 0.01
Mean Sphericity	0.75 ± 0.01	0.75 ± 0.02	0.76 ± 0.01	0.75 ± 0.01	0.74 ± 0.01
Filamentous algal cover (%)	6.5 ± 4.3	3.2 ± 3.1	4.1 ± 0.8	6.2 ± 4.8	10.9 ± 2.3
Encrusting algal cover (%)	14.0 ± 19.7	1.1 ± 2.1	3.0 ± 5.7	1.2 ± 1.6	40.6 ± 11.8
Ann. Av. Temperature (°C)	20.65 ± 0.76	21.25 ± 1.32	20.41 ± 0.38	20.27 ± 0.28	20.58 ± 0.18
Ann. Av. Salinity (psu)	37.94 ± 0.13	37.87 ± 0.22	37.97 ± 0.07	38.00 ± 0.06	37.95 ± 0.11
Ann. Av. Dissolved Oxygen (%)	107.2 ± 5.1	105.1 ± 7.6	107.7 ± 5.9	107.3 ± 3.9	108.7 ± 2.9
Ann. Av. Turbidity (NTU)	0.52 ± 0.58	0.44 ± 0.20	0.74 ± 1.28	0.37 ± 0.22	0.55 ± 0.30
Ann. Av. [Nitrate] (µg/L)	78.4 ± 115	156.0 ± 205.6	46.9 ± 50.5	90.6 ± 55.5	26.8 ± 13.4
Ann. Av. [Phosphate] (µg/L)	2.6 ± 1.3	2.8 ± 1.1	2.5 ± 0.3	3.4 ± 2.4	1.9 ± 0.2

The upper pebbly stratum generally consisted of around two to three layers of pebbles, thus having an overall thickness in the 5–10 cm range. These pebbles mostly fell within the 16–32 or 32–64 mm size classes. Some variation in mean pebble size was evident between sites, but there was no consistent pattern of variation among the four pebble-bed types; measures of mean moment of inertia followed the same pattern as those of mean pebble size. On the other hand, harbour sites consistently had the highest proportion of sand and silt and algal cover, while shallow sites had the lowest content of fine sediment and algal cover. Shallow sites also had the highest total surface area, reflecting the slightly thicker pebble beds present at these sites.

In terms of particle-shape characteristics, elongation and flatness were within the 0.6–0.8 range for nearly all sites, and thus the pebbles were classified as “slightly elongate” and “slightly flat” according to Blott and Pye’s (2008) classification system. Similarly, all fifteen beds had “rounded” pebbles, although this category includes all roundness values between 0.5 and 1.0 since visual differentiation between the higher roundness scales is difficult (Blott and Pye, 2008). All sites had pebbles falling into the “moderately spherical” category, which includes particles with sphericities of 0.632–0.775 according to Riley’s (1941) method.

With regards to the physico-chemical parameters, there was minimal variation in annual average values for salinity, dissolved oxygen, turbidity and phosphate content between sites. Similar temperature values were also recorded at the different sites except at il-Hofra, where the presence of a power station coolant water outflow close to the pebble bed resulted in temperature being around 3°C higher at this site; this also led to the slightly higher average temperature for the ‘shallow’ group of sites shown in Table 2.2. Some variation in levels of nitrate were also noted, with shallow sites having the highest values overall, although all values were within the range that would be expected of local inshore waters (Axiak, 2004).

CLUSTER analysis grouped sites into three main clusters (Figure 2.3) according to their physical characteristics; these were also reflected in the ordination pattern obtained via PCA (Figure 2.4). Cluster C included the six harbour sites, while nearly all the sites with seagrass or creek pebble-bed types grouped together in Cluster B. Cluster A comprised three of the five shallow sites, together with Mistra, which was the shallowest site with a seagrass pebble-bed type, while the il-Hofra site was an outlier. The PCA ordination pattern suggests that Cluster B was intermediate between the other two clusters given their positioning along the first principle component axis.

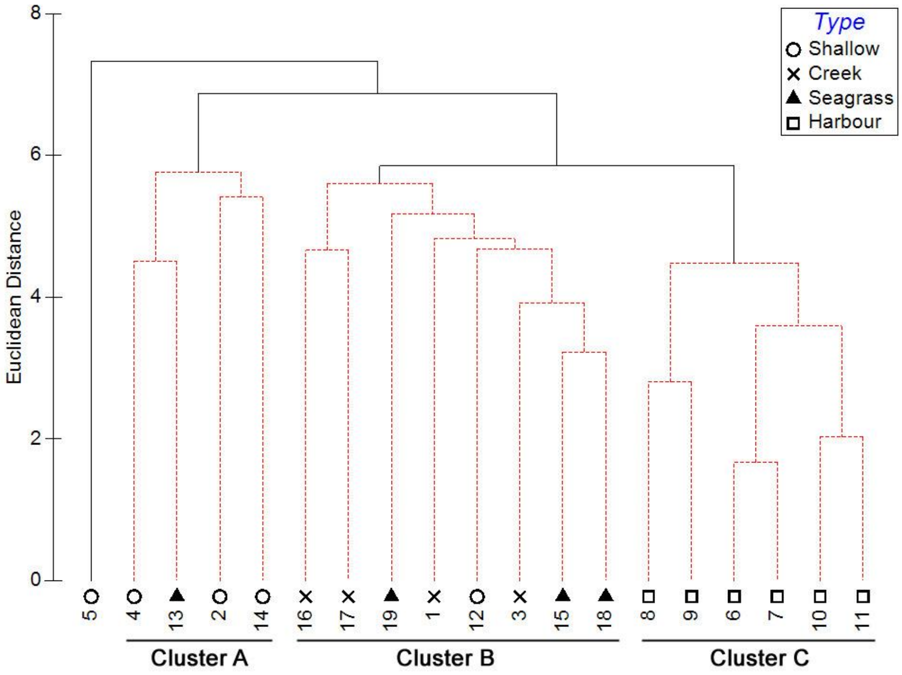

Figure 2.3 Dendrogram obtained by CLUSTER analysis utilising group-average hierarchical clustering, based on a Euclidean distance resemblance matrix calculated on standardised environmental variables; sites which were not considered to be statistically different by the SIMPROF routine are shown connected by dotted red lines.

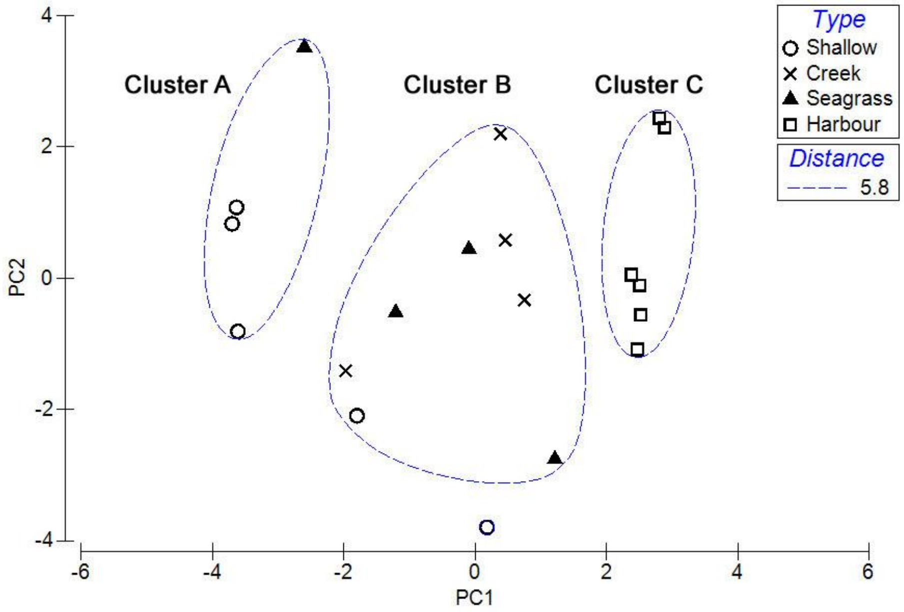

Figure 2.4 PCA ordination plot of the nineteen sites in terms of their (standardised) environmental variables. Dashed blue lines indicate the site groupings as identified by CLUSTER analysis (Figure 2.3).

Multivariate multiple regression showed a similar relationship between biological and environmental data when using Bray-Curtis similarity or χ^2 distance as the resemblance measure for biotic data (Table 2.3). When considering predictor variables individually, both analyses indicated a significant relationship for the same set of physical variables, while the percentage of variance in species data explained by the variables according to the two analyses were highly correlated (Pearson $r = 0.95$). In both cases, the sand and silt content was the physical parameter that accounted for the highest variance in species data, closely followed by the coverage of the pebble beds and, to a slightly lesser extent, the percentage cover of algae on the pebbles and the depth of the beds.

Table 2.3 Results of DistLM analysis based on the adjusted-R^2 criterion for (a) each variable taken individually (ignoring other variables), (b) and (c) forward-selection of variables, where the amount explained by each variable added to the model is conditional on variables already in the model (i.e. those variables listed above it); only variables up to the first highly non-significant *p*-value (>0.1) are included in sequential fitting. Separate sets of results are shown for analyses performed using Bray-Curtis similarity (a, b) or χ^2 distance (a, c) to measure variance in biotic data. % var: percentage of variance in species data explained by that variable; cum. %: cumulative percentage of variance explained.

(a) Variables taken individually

Variable	**Bray-Curtis similarity**			**χ^2 distance**		
	% var	**Pseudo-F**	**P**	**% var**	**Pseudo-F**	**P**
Depth	17.6	3.635	**0.003**	13.4	2.642	**0.001**
Layer Thickness	9.6	1.799	0.072	8.2	1.514	0.055
Patch Area	25.6	5.865	**0.001**	15.2	3.039	**0.001**
Mean Pebble Size	7.8	1.434	0.166	7.2	1.327	0.155
% Sand & Silt	26.7	6.181	**0.001**	16.8	3.422	**0.001**
Mean Elongation	2.5	0.435	0.978	3.0	0.529	0.992
Mean Flatness	5.3	0.944	0.435	6.6	1.208	0.220
Mean Roundness	6.9	1.255	0.233	6.5	1.191	0.210
Mean Sphericity	6.6	1.196	0.257	6.8	1.249	0.164
Mean Moment of Inertia	11.6	2.229	**0.033**	9.2	1.724	**0.018**
Total Surface Area	11.7	2.261	**0.027**	10.1	1.910	**0.016**
% Filamentous Algae	18.3	3.802	**0.001**	11.9	2.300	**0.006**
% Encrusting Algae	20.4	4.366	**0.001**	12.7	2.482	**0.001**
Ann. Av. Temperature	7.0	1.274	0.202	9.6	1.804	0.057
Ann. Av. Salinity	4.5	0.797	0.646	6.4	1.166	0.256
Ann. Av. DO	6.7	1.221	0.258	6.3	1.137	0.272
Ann. Av. Turbidity	6.5	1.186	0.280	5.5	0.994	0.475
Ann. Av. Nitrate	13.7	2.696	**0.018**	9.5	1.778	**0.018**
Ann. Av. Phosphate	16.4	3.340	**0.006**	10.7	2.038	**0.004**

Table 2.3 (continued)

(b) Variables fitted sequentially, based on Bray-Curtis similarity

Variable	**Pseudo-F**	**P**	**% var**	**cum %**
% Sand & Silt	6.181	**0.001**	26.7	26.7
Patch Area	2.495	**0.001**	9.9	36.6
Depth	2.001	**0.009**	7.5	44.0
Layer Thickness	1.878	**0.032**	6.6	50.6
Mean Pebble Size	1.474	0.103	5.0	55.7

(c) Variables fitted sequentially, based on χ^2 distance

Variable	**Pseudo-F**	**P**	**% var**	**cum %**
% Sand & Silt	3.422	**0.001**	16.8	16.8
Ann. Av. Temperature	1.921	**0.046**	8.9	25.7
Patch Area	1.585	**0.034**	7.1	32.8
Ann. Av. DO	1.291	0.149	5.7	38.5

When the environmental factors were added sequentially to build a parsimonious model correlated to the variation in biotic composition, the first chosen factor was the percentage sand and silt content. This was followed by patch area, depth and layer thickness when using Bray-Curtis similarity, with the four factors explaining 50.6% of the variation in assemblage structure. Using χ^2 distance, the annual average temperature and patch area were fitted after the sand and silt content, with the three variables together accounting for 32.8% of the biotic variation. Addition of further parameters to the models resulted in non-significant *p*-values, and once a large non-significant *p*-value has been encountered there is little justification to include this parameter or any of the other subsequent ones (Anderson *et al.*, 2008).

Db-RDA ordination plots of the sites based on biotic data, but constrained by their correlation with environmental variables are shown in Figures 2.5 and 2.6. Only the physical factors selected in the DistLM models were included. The first two axes account for 80-83% of the fitted variation in either case, but only 27% (χ^2 distance; Figure 2.6) to 40% (Bray-Curtis similarity; Figure 2.5) of the total variation in assemblage structure. Both ordination plots show separation of the harbour, seagrass/creek, and shallow pebble-bed types along the first db-RDA axis, with the percentage sand and silt content being the variable most strongly correlated with this axis. Patch area tends to separate the harbour sites from the rest. Layer thickness and depth were related to differences between the shallow sites and the seagrass/creek sites in the Bray-Curtis similarity based analysis. This was less evident in the db-RDA plot produced on the basis of the χ^2 distance DistLM analysis, where the inclusion of annual average temperature as a physical variable separated the il-Hofra sampling site from the rest.

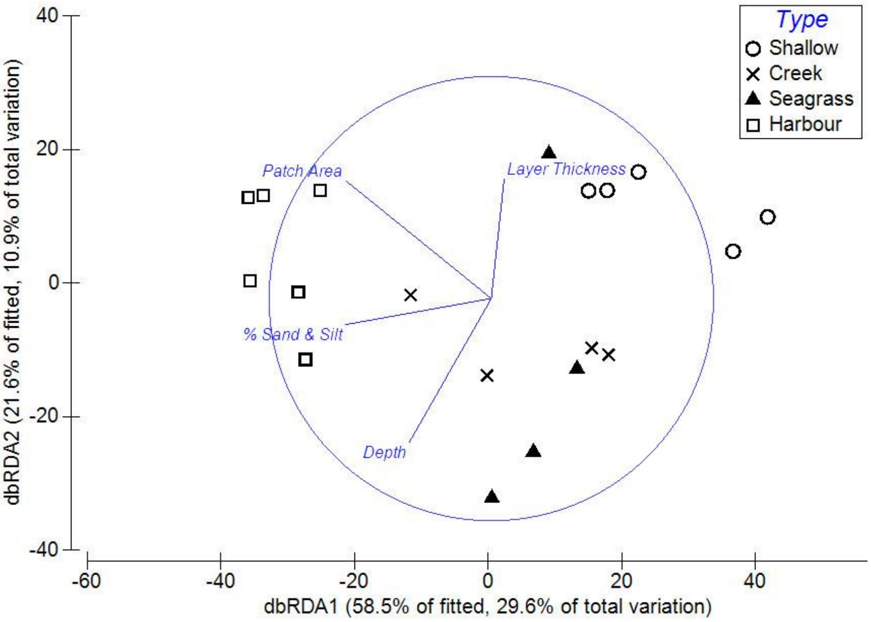

Figure 2.5 Distance-based RDA ordination relating environmental variables to the biotic data, performed using Bray-Curtis similarity of square-root transformed species counts, with vector projections of the physical factors selected by the DistLM routine (Table 2.3(b)). Length and direction of the vectors represent the strength and direction of the relationship.

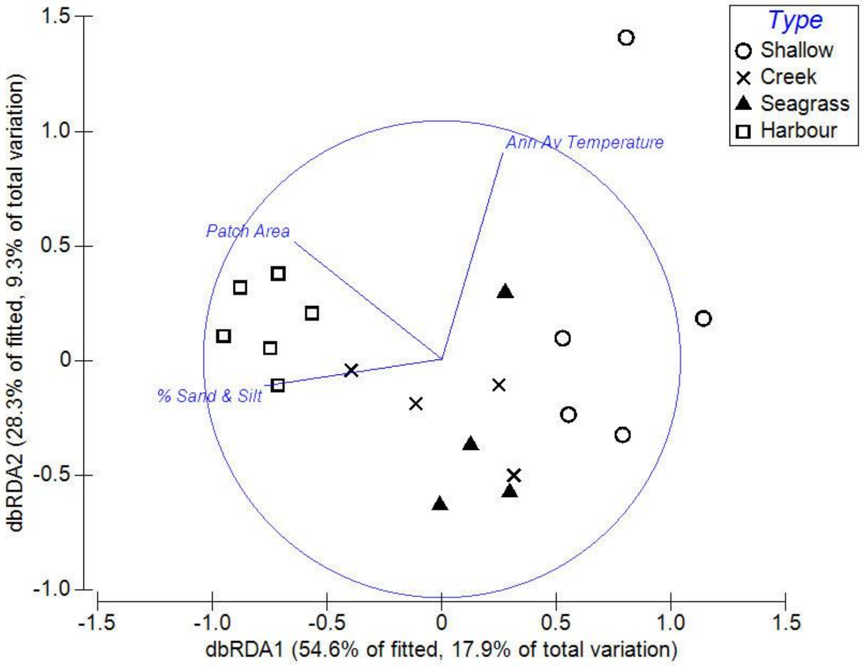

Figure 2.6 Distance-based RDA ordination relating environmental variables to the biotic data, performed using χ^2 distance of square-root transformed species counts, with vector projections of the physical factors selected by the DistLM routine (Table 2.3(c)). Length and direction of the vectors represent the strength and direction of the relationship.

2.4 Discussion

Preliminary classification of pebble beds found in the Maltese Islands based on seascape features observed in the field yielded four pebble-bed categories, but analysis of the measured physical parameters indicated that two of these categories shared very similar environmental attributes. Thus, three different pebble-bed types were characterised in the present study.

The first type, here referred to as ‘shallow’ beds, fits the description of the pebble-bed habitat supporting the Mediterranean biocoenosis of infralittoral pebbles given in habitat manuals (Pérès and Picard, 1964; Pérès, 1967; Bellan-Santini, 1985; Bellan-Santini *et al.*, 1994). Such beds may be physically similar to the British biotope of highly mobile sublittoral shingle (cobbles and pebbles) described by Connor *et al*. (2004), which occurs in marine inlets with very strong tidal currents or in wave exposed open coast environments. This British biotope is known to occur at depths of up to 50 m, whereas the Mediterranean biocoenosis of infralittoral pebbles occurs in very shallow depths that rarely exceed a metre, possibly because pebble beds found in deeper waters in the Mediterranean are less mobile due to the lack of strong tidal currents. Both habitats are thought to be impoverished as a result of physical disturbance (Bellan-Santini, 1985; Connor *et al*., 2004; but see Chapters 3 and 4).

The second pebble-bed type occurred at depths of 2–12 m, either within creeks or in the inter-matte region of *Posidonia oceanica* beds. These pebble beds had a marginally higher cover of filamentous and coralline algae than those found in shallower sites and a higher sand and silt content. Such habitats are not included in current benthic habitat classification schemes for the Mediterranean. For instance, seagrass inter-matte spaces are described as containing fine to coarse sand and gravels (Pérès, 1967; Bellan-Santini *et al*., 2002) and the existence of patches of pebbles, which represent a different biocoenosis to fine or coarse sand, is not acknowledged. However, this is not the first time that accumulations of pebbles have been noted among *P. oceanica* beds (Colantoni *et al*., 1982) or in creeks (Linnane *et al*., 2001), indicating that such pebble-bed types are more widespread in the Mediterranean.

Accumulations of pebbles in harbour environments formed the third pebble-bed type characterised during the present study. These beds were characterised by a higher content of fine particles belonging to the sand and silt fraction and more extensive cover by encrusting coralline algae. They also covered comparatively larger areas than other bed types because they occurred as bands parallel to the shore, rather than as patches with similar major and minor axis lengths, as found within narrow creeks or coves or amongst seagrass beds.

It is interesting to note that all the pebble beds observed in the Maltese Islands occurred in coastal inlets or embayments, with no such habitats being present along the open coast. This is also true for the locations studied by Osman (1977), Davis and Wilce (1987a, 1987b), Scheibling and Raymond (1990), Linnane *et al*. (2003) and Scheibling *et al*. (2009a, 2009b), although locations with cobble habitats along an open coast do exist (Lieberman *et al*., 1979, 1984; Robinson and Tully, 2000a, 2000b). However, most of these studies focused on areas with sublittoral cobble beds, where the larger cobbles were dominated by erect algae due to their lower susceptibility to overturn as a result of physical disturbance.

Small boulders with erect algae were also observed within, or close to, the Maltese pebble beds, but the pebbles themselves were not colonised by macrophytes – neither in winter when the preliminary surveys were carried out, nor in summer when actual sampling was undertaken. This suggests that they are unstable and overturn regularly. Physical disturbance via wave action is probably the major cause of pebble overturn during winter months, but may play a minor role during prolonged periods of calm weather that occur in summer, especially for the sheltered harbour sites. Field observations made whilst sampling indicated that bioturbation, especially by the striped red mullet *Mullus surmuletus*, may be an important cause of disturbance in the deeper sites. Anthropogenic disturbance also occurs: most of the shallow sites are popular bathing spots and the pebble beds are within wading depth, while divers frequent the harbour area to collect the edible warty venus *Venus verrucosa* from beneath the pebble layer and in so doing disturb the pebble bed.

Although CLUSTER analysis distinguished three different pebble-bed types in the Maltese Islands, close inspection of the various environmental attributes (Table 2.2) suggests that for certain parameters the differences are rather small and probably biologically insignificant. This applies mainly to the pebble-shape characteristics and water physico-chemical elements. Indeed, multivariate multiple regression showed that these were not correlated with changes in biotic composition across sites. One of the main factors which did differ among the three pebble-bed types was the sand and silt content. In sedimentary habitats, the type of sediment is mainly determined by hydrodynamic regime; coarser sediment is typically found in more exposed locations (Connor *et al*., 2004), as a result of selective entrainment of smaller particles in higher energy environments (Parker and Klingeman, 1982).

In microtidal areas such as the Maltese Islands (maximum tidal range: 20 cm; Drago, 2009), the hydrodynamic regime at a given site ultimately depends on its exposure to wave action and on the depth (due to wave energy depth attenuation). This explains why shallow sites had the lowest content of sand and silt and largest mean pebble size, while pebble beds located in harbours had the highest content of fine sediment. Nonetheless, all three pebble-bed types exhibited vertical stratification, with a pebble layer found at the surface and finer sediment below.

Stratification may be partly due to the selective fine particle entrainment, which renders the surface layer coarser than the subsurface material. It could also result from vertical winnowing (Parker and Klingeman, 1982), which involves loss of fine material to the subsurface by such material falling through the interstitial cavities between the pebbles as these are disturbed by water motion or bioturbation. Such mechanisms enable the pebbles to persist in an unburied state. Stratification can also have important biological consequences since benthic community structure is often related to sediment textural characteristics (Gray, 1974; Anderson, 2008), which could lead to different species groups being present in the different strata at a given site.

The DistLM analyses indicated that the sand and silt content of pebble beds was the main physical factor correlated with the structure of the biotic assemblage they support, probably because it is a good proxy for the hydrodynamic regime present at a site. It was initially hypothesized that some pebble-shape characteristics, notably roundness, would also depend on the hydrodynamics of the site and thus show a similar pattern to fine particle content. However, roundness may be a less suitable proxy measure because it also depends on other factors such as pebble hardness. It is also possible that pebbles were rounded under a previous hydrodynamic regime that now no longer exists.

In addition, the higher percentage cover of coralline algae observed on pebbles found in more sheltered locations may have increased their roundness to levels comparable with those found in shallow sites. The percentage cover of encrusting algae was actually itself one of the parameters that were most correlated with the biotic patterns, but was not included in either of the DistLM parsimonious models. Since coralline algal cover was also correlated with the sand and silt content (Pearson $r = 0.81$), it may have accounted for little of the residual variation in assemblage structure once the fine particle content had already been included in the models.

The other physical variables included in the DistLM models were patch area, depth, pebble layer thickness and water temperature, although only patch area was included in both models. Temperature mainly distinguished a single site (il-Hofra) from the rest and was clearly due to the power station water coolant outflow at this site leading to increased temperatures. Sea surface temperature may therefore be of relevance wherever local thermal hotspots occur, whether anthropogenic (e.g. thermal effluent from power stations and other industrial installations) or natural (e.g. shallow-water hydrothermal vent areas; see Dando *et al*., 1999 for a review).

Depth can be a proxy for several other factors, including wave energy, light intensity and phytoplankton concentration, all of which may have an influence on benthic assemblages. The pebble layer thickness is a measure of habitat complexity. On its own, this factor explained less than 10% of the biotic variation but could explain almost 7% of the variation over and above that accounted for by the other parameters already in the model.

The inclusion of coverage is interesting given that the pebble patches are essentially habitat islands surrounded by other types of habitat and, according to the classic theory of island biogeography, island area is one of the main factors influencing the occurrence and abundance of species in islands (MacArthur and Wilson, 1967). This may be due to patch area *per se*, but can also result from its correlation with other factors that directly regulate the community structure (Ricklefs and Lovette, 1999). In the present context, pebble patch area was one of the main factors distinguishing harbour sites from those with other pebble-bed types, but it is not possible to determine if any differences in the harbour fauna were due to the larger patch area itself or other factors.

This highlights an important consideration: while the present results suggest that pebble-bed assemblage structure is correlated with certain environmental factors, these cannot be interpreted as being causative, given that none of the variables have been manipulated or controlled for in a manner that allows such inferences (Anderson *et al*., 2008). Nonetheless, these results still provide useful insights about the relationship between the physical nature of the pebble habitat and the biological assemblage it supports. The constrained ordination plots produced by db-RDA both showed separation of the three pebble-bed types previously identified by CLUSTER analysis of physical variables (i.e. the ‘shallow’, ‘seagrass/creek’ and ‘harbour’ types), suggesting that the biotic assemblage may be responding to a complex set of environmental variables associated with these habitat types.

In such a situation, changes to the physical environment can lead to a significant impact on the assemblage structure and composition. In particular, the results of this study indicate that pebble-bed macrofaunal assemblages are sensitive to changes in hydrodynamic conditions and sediment loads, to increases in water temperature, to alterations to the stratified structure of the pebble beds, and to fragmentation of the habitat patches. This information will enable better assessment of the potential impacts on pebble-bed assemblages caused by anthropogenic disturbance such as coastal development.

Chapter 3:

Biological characterisation of infralittoral pebble beds in the Maltese Islands

Chapter 3

Biological characterisation of infralittoral pebble beds in the Maltese Islands

Abstract

Characterisation of benthic habitats is necessary to develop ecologically meaningful habitat classification schemes and identify priority habitats for conservation, especially since benthic habitats are increasingly being used as surrogate measures of biodiversity in the design of marine protected areas (MPAs), yet many marine habitats remain inadequately studied. One such habitat is the Mediterranean biocoenosis of infralittoral pebbles. The present study on pebble beds in the Maltese Islands characterised three distinct pebble-bed assemblages, showing that these are not homogeneous habitats. Shallow pebble assemblages matched the descriptions of the Mediterranean biocoenosis of infralittoral pebbles given in habitat manuals, but deeper assemblages occurring either in creeks and seagrass beds or within harbours have not been previously described. Suggestions for amendments to existing benthic habitat classification schemes are therefore provided. Contrary to the prevailing view that pebble beds are impoverished, the present findings indicate that they are rich and diverse habitats. This, together with the overall rarity of such habitats (in terms of coverage), suggests that the occurrence of pebble-bed habitats should be taken into consideration when siting MPAs.

3.1 Introduction

Establishing networks of marine protected areas (MPAs) is seen as a mechanism for conservation and sustainable management of marine biodiversity in the coastal zone, especially where tight control of human activities is necessary (Allison *et al*., 1998; Banks *et al*., 2005). In Europe, establishment of protected areas as management tools is a requirement under the EU ‘Habitats Directive’ (HD, 92/43/EEC) and ‘Marine Strategy Framework Directive’ (MSFD, 2008/56/EC). Annex I of the HD lists habitats whose conservation requires designation of protected areas, but marine habitats are not well represented with only eight categories[1] being included. A more comprehensive classification of Mediterranean marine habitats (UNEP-MAP-RAC/SPA, 2006a) has been developed by the Regional Activity Centre for Specially Protected Areas (RAC/SPA) within the ambit of the Barcelona Convention. The RAC/SPA subsequently produced a reference list of priority habitats to guide selection of sites of conservation interest (UNEP-MAP-RAC/SPA, 2006b).

Designing MPA networks on the basis of benthic habitat maps rests on the assumption that benthic habitats can act as surrogate measures of biodiversity (e.g. Ward *et al*., 1999). In the absence of detailed data on the distribution and ecology of species within a region, habitat mapping enables decisions on the locations of marine reserves to be made reliably. However, this approach is still dependent on ecological knowledge of the habitats (Howell *et al*., 2010). For instance, mapping exercises often rely heavily on abiotic variables to delineate habitat borders (Brown *et al*., 2011); before such maps can be used to guide MPA design, it is necessary to ascertain whether the defined

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[1] Although these are referred to as “habitats” in the HD, most are actually complexes of habitats; for instance “large shallow inlets and bays” may incorporate a number of different habitat types among those included in the European Nature Information System (EUNIS) database.

habitats correlate well with, and can therefore predict, the patterns of biological distributions (Stevens and Connolly, 2004). Ecological knowledge is also necessary to establish whether some of these habitats have a higher intrinsic biological value, in order to draw up lists of priority habitats (DFO, 2004; Derous *et al*., 2007). This is particularly relevant to assemblages having rare species, given that these species are unlikely to be detected if their habitats are not extensively studied. Baseline information on the species assemblages characterising different habitat types is also vital for assessment of the effectiveness of MPAs, since any future shift in the species composition of assemblages cannot be evaluated without knowledge of the present state (Kipson *et al*., 2011).

Many marine habitats have been inadequately studied and consequently marine reserve boundaries have often been set on the basis of *ad hoc* evaluations with minimal ecological considerations (Fraschetti *et al*., 2005; Dauvin *et al*., 2008). In the Mediterranean, one poorly-studied habitat is that of accumulations of infralittoral pebbles. Almost no information about this biocoenosis is provided in the synthetic account of Bellan-Santini *et al*. (1994), or even in recent interpretive manuals for the identification of Mediterranean biocoenoses (e.g. Bellan-Santini *et al*., 2002; Relini and Giaccone, 2009). Indeed, the original description by Pérès and Picard (1964) appears to be the most complete available to date. Here infralittoral pebbles are described as occurring in wave-exposed rocky coves at depths of no more than a few decimetres. On this basis, the UNEP-MAP-RAC/SPA (2006a) benthic habitat classification scheme includes only one category for this habitat (III.4.1 Biocoenosis of infralittoral pebbles). This biocoenosis is considered to be impoverished (Bellan-Santini, 1985) and has therefore not been considered as a priority habitat (UNEP-MAP-RAC/SPA, 2006b).

In the European Nature Information System (EUNIS) habitat classification scheme infralittoral pebble beds fall under habitat code A5.13 (Infralittoral coarse sediment), which also encompasses coarse sediment biotopes. A total of eighteen habitat sub-types are classified under this category, but most are derived from classifications of marine habitats in Britain and Ireland and in the Black Sea; it is impossible to tell whether analogous assemblages are present in the Mediterranean, given the limited current knowledge. In addition, the available descriptions for the Mediterranean infralittoral pebble habitat appear to be incomplete. For instance, in their search for the early benthic phase of the European lobster *Homarus gammarus*, Linnane *et al*. (2001) included a sampling site at Elba Island, Italy, where the substratum consisted of cobbles on a sand and shingle bottom at depths of 9–13 m. Similarly, Evans *et al*. (2010, 2011) listed accumulations of cobble and pebbles at depths of 5–12 m in Marsamxett, Malta as the preferred habitat of the Maltese top-shell *Gibbula nivosa*. Thus, neither site fits into the description of pebbles occurring at depths of up to a few decimetres as given by Pérès and Picard (1964) and subsequent authors.

Furthermore, a total of 20 decapod species were recorded at Elba Island (Linnane *et al*., 2001), while 69 species of molluscs were collected from Marsamxett (Evans, 2007), suggesting that these pebble beds may not be as impoverished as claimed by Bellan-Santini (1985), although she does concede that they have been very poorly studied. In addition, the Maltese top-shell, a marine point-endemic found solely in Malta and listed in Annex II of the HD, is currently known to occur only in infralittoral pebble habitats (Evans *et al*., 2010, 2011) and these may therefore be deemed priority habitats within the local context.

A re-assessment of the classification and diversity of Mediterranean infralittoral pebble habitats based on biological sampling is therefore due. The Maltese Islands' location at the biogeographic boundary between the western and eastern Mediterranean bioregions (Bianchi, 2007) makes them ideally suited for such preliminary characterisation of Mediterranean habitats given that the Maltese marine biota has elements from practically all the biogeographic regions of the Mediterranean. The present study was therefore carried out to characterise the infralittoral pebble-bed assemblages of the Maltese Islands with the aim of providing: (i) an updated classification scheme for this habitat derived from analysis of species-assemblage distributions, which therefore meets the requirements for benthic mapping, and (ii) an assessment of the diversity of pebble-bed assemblages, both of which can aid MPA design. Physical characterisation of these habitats is treated in Chapter 2.

3.2 Material and methods

A preliminary survey was carried out by snorkelling along the low-lying coasts of the Maltese Islands in order to map the presence of infralittoral pebble beds. Surveying was carried out during January–March 2011. A total of 15 locations were chosen for further study, based on the following selection criteria: (i) the area consisted predominantly of pebbles (stones having a diameter of 2–10 cm following the UNEP-MAP-RAC/SPA [2006a] criteria), which lacked a covering of erect macrophytes; (ii) an area of at least 25 m^2 of pebble habitat was present at a depth of more than 0.5 m below chart datum. Areas with cobbles or small boulders that had a permanent cover of erect algae were excluded because they are considered to belong to a different biocoenosis – that of infralittoral algae (e.g. Pérès and Picard, 1964; Bellan-Santini *et al.*, 1994).

Since most of the locations had a small coverage (typically <50 m^2), it was not possible to nest more than one site within a single location without leading to pseudoreplication (Hurlbert, 1984); on the other hand the relatively large beds (>1000 m^2) present at Tigné and Ta’ Xbiex and presence of two separate beds at Manoel Island and Wied ix-Xoqqa permitted use of two sites at each of these four locations. Therefore a total of nineteen sites were surveyed in the present study (see Figure 3.1 and Table 3.1).

Four replicate samples were collected from each of the 19 sites; their position was selected at random, but no samples were collected towards the edges of the pebble beds to avoid edge effects (e.g. Todd and Turner, 1986). Sampling was carried out using a 0.1 m^2 circular sampler (modified from Borg *et al.*, 2002) which was pushed into the substratum to delineate the sampling area and prevent escape of motile invertebrates. Cobbles and pebbles were hand-collected and transferred to a 0.5 mm mesh bag, while a small fine-mesh hand net (1 mm mesh size) was used to scoop the basal layer of finer granules. A suction sampler was simultaneously employed to reduce the risk of missing highly-motile organisms, thus ensuring quantitative samples were collected. Sampling depth was also measured using a digital depth gauge. All fieldwork was carried out between July and September 2011.

Samples were subsequently sorted in the laboratory, separating the macrofauna (retained by a 0.5 mm sieve; e.g. Castelli *et al.*, 2003) into major taxonomic groups. The biota were identified to the lowest taxon possible and enumerated to construct a taxon-abundance matrix and obtain estimates of number of taxa and abundance per sample.

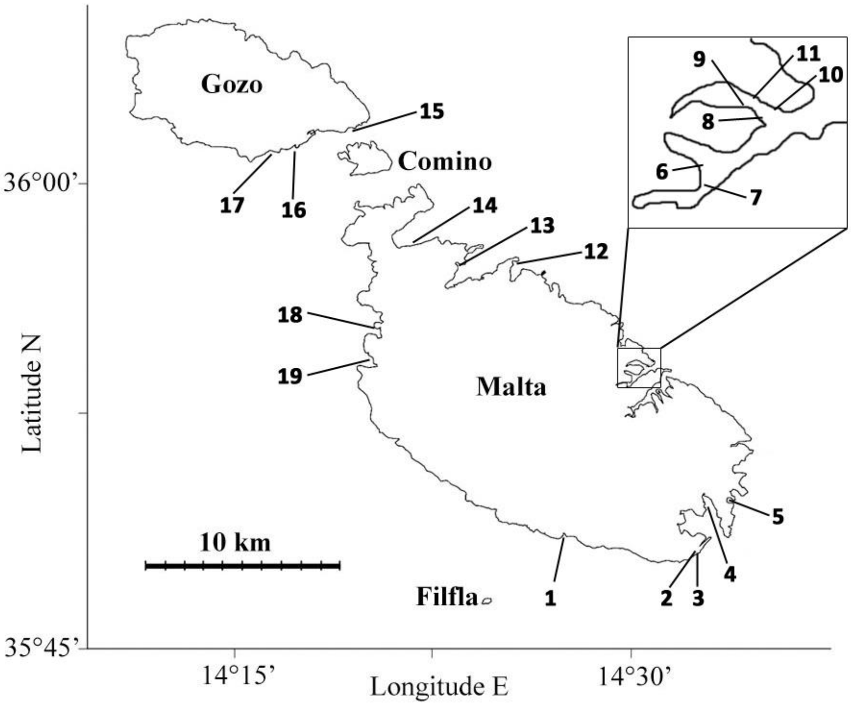

Figure 3.1 Map of the Maltese Islands indicating the position of the nineteen sampling sites used in the present study. Inset shows enlarged view of Marsamxett Harbour. Site numbers correspond to those listed in Table 3.1.

Table 3.1 Pebble-bed sites used in the present study as categorised into different bed types based on seascape features in the field (see Results), together with their respective geographical coordinates (WGS84 datum), sampling depth and physical description.

Bed type	Site	Coordinates (Latitude/Longitude)		Depth (m)	Description
(i) Shallow	2. Wied ix-Xoqqa A	35° 48.684'N	4° 32.327'E	0.7	Smooth pebbles generally devoid of algal cover with minimal sand and silt content within the upper layers of the bed
	4. Marsaxlokk	35° 50.030'N	14° 32.710'E	0.8	
	5. Hofra z-Zghira	35° 50.281'N	14° 33.597'E	0.8	
	12. Qawra	35° 57.559'N	14° 25.571'E	1.4	
	14. Tunnara	35° 58.010'N	14° 21.441'E	1.0	
(ii) Seagrass	13. Mistra	35° 57.462'N	14° 23.444'E	1.2	Top layer of pebbles often with a cover of filamentous turf algae at the surface; greater content of sand and silt within the bed than in shallow sites
	15. Hondoq ir-Rummien	36° 01.672'N	14° 19.509'E	6.7	
	18. Gnejna	35° 55.423'N	14° 20.546'E	6.3	
	19. Fomm ir-Rih	35° 54.711'N	14° 20.131'E	12.0	
(iii) Creek	1. Wied iz-Zurrieq	35° 49.238'N	14° 27.119'E	6.9	
	3. Wied ix-Xoqqa B	35° 48.623'N	14° 32.361'E	6.0	
	16. Xatt l-Ahmar	36° 01.153'N	14° 17.292'E	2.3	
	17. Mgarr ix-Xini	36° 01.177'N	14° 16.323'E	6.7	
(iv) Harbour	6. Ta' Xbiex A	35° 53.987'N	14° 30.068'E	4.9	Pebbles with relatively high cover of coralline algae, giving rise to rhodoliths; highest content of sand and silt within the bed
	7. Ta' Xbiex B	35° 53.904'N	14° 30.054'E	5.6	
	8. Manoel Island A	35° 54.253'N	14° 30.462'E	8.2	
	9. Manoel Island B	35° 54.319'N	14° 30.382'E	6.5	
	10. Tigné A	35° 54.368'N	14° 30.593'E	7.5	
	11. Tigné B	35° 54.410'N	14° 30.467'E	10.1	

The different sites were assigned to one of four habitat categories (‘bed types’; see Table 3.1) based on seascape features. These included pebble beds occurring (i) in very shallow waters (<2 m) within small rocky coves, (ii) in slightly deeper regions within creeks (with only ephemeral freshwater input), (iii) within inter-matte regions of reticulate *Posidonia oceanica* beds, or (iv) in harbour environments; these are hereafter referred to as ‘shallow’, ‘creek’, ‘seagrass’ and ‘harbour’ pebble-bed types respectively. Sites were classified into these four categories as indicated in Table 3.1, while their spatial distribution is shown in Figure 3.1. Harbour sites were located in close proximity to one another, which could contribute to similarity in their biotic composition. This was not the case for sites with shallow, seagrass or creek pebble-bed types, where any similarity in species assemblages among sites must therefore be due to the nature of the habitat itself.

Physical characterisation of these four bed types indicated that they can be reduced to three categories, because ‘seagrass’ and ‘creek’ pebble-bed types had similar environmental characteristics (Chapter 2). However, biological features may not necessarily reflect the physical attributes. In addition, these two bed types were bordered by different adjacent habitats, which could also influence the species composition of the pebble-bed assemblages. Therefore, analysis of the biological data was initially based on the original four habitat categories.

Two complementary approaches were used in order to determine whether the infralittoral pebble-bed habitats could be further subdivided into distinct sub-types on the basis of their biotic components. First, the ability of the four pebble-bed types to account for variation in species assemblages was tested using permutational multivariate Analysis of Variance (PERMANOVA; Anderson, 2001). The analysis consisted of a 2-way model with ‘Bed type’ as a fixed factor and ‘Site’ as a random factor nested in ‘Bed type’.

Secondly, agglomerative hierarchical cluster analysis with group-average linkage was used to group sites according to their biotic composition, without taking into consideration any *a priori* classification. This initially resulted in clustering of replicates by site and the analysis was therefore repeated using mean species abundances per site to obtain a more readily interpretable dendrogram. Similarity Profile (SIMPROF; Clarke *et al.*, 2008) permutation tests (at 1% significance level) were carried out at every node of the completed dendrogram to determine whether there was significant sub-group structure within clusters. Non-metric multidimensional scaling (nMDS) was also performed to visualise the community pattern.

These multivariate analyses were carried out on a Bray-Curtis similarity resemblance matrix calculated on square-root transformed data. The Bray-Curtis coefficient was chosen since it satisfies a number of biologically desirable criteria, providing an intuitive measure of ecological similarity (Clarke, 1993; Legendre and Legendre, 1998), but it tends to be unduly dominated by counts for highly-abundant species. A mild square-root transformation was therefore applied to down-weight the importance of such highly-abundant species. This allows species of intermediate abundance to also contribute to the similarity calculations, while not providing too much weight to rarer species (Clarke and Green, 1988) since this would not be useful for identification of assemblages for mapping efforts (Howell *et al.*, 2010). Pebble-bed assemblage types were defined in accordance with the outcome of the PERMANOVA and cluster analyses, and the characteristic and discriminating species for each type were determined using Similarity Percentage analysis (SIMPER; Clarke, 1993), enabling description of the assemblages in terms of their biotic components.

To assess the diversity of pebble-bed assemblages, a set of univariate indices were calculated for each site and used to obtain mean values per site for each assemblage type. These included the observed taxon richness (S_{obs}) and estimated total richness (S_{est}), total abundance (*TA*), diversity (Hill numbers ${}^{q}D$), evenness (${}^{1}D/S_{\mathrm{obs}}$) and relative evenness (Pielou *J’*). Following the recommendations of Foggo *et al*. (2003), the *Chao1* estimator (Chao, 1984) was used to estimate S_{est}, while choice of evenness measures was based on the rationale of Jost (2010). Hill numbers measure “true diversity” (*sensu* Jost, 2006) and differ among themselves only in the value of the exponent *q*, which controls the weight attributed to common species. They were calculated for values of *q* ranging from 0 to 4, enabling the construction of diversity profiles (Tóthmérész, 1995).

These indices reflect differences in community structure, but since functionally redundant species tend to be taxonomically related (Clarke and Warwick, 1998a), indices that take species’ relatedness into account provide a broader view of biodiversity that potentially also includes functional aspects (Clarke and Warwick, 1998b; Somerfield *et al*., 2008). Thus, taxonomic diversity (Δ), taxonomic distinctness (Δ^*) (Warwick and Clarke, 1995) and average variation in taxonomic distinctness (Λ^+) (Clarke and Warwick, 2001) indices were also estimated. Comparisons between assemblage types were carried out via univariate PERMANOVA based on Euclidean distance; this is analogous to traditional ANOVA except that the null distribution of the test statistic is produced by permutation, thus avoiding the usual assumptions of parametric tests (Anderson, 2001).

One attribute of relevance to conservation is the number of unique taxa a particular habitat contains, but using raw counts of shared ($S_{12(obs)}$) and unique (U_{obs}) taxa ignores the possibility that some taxa may have been recorded from only one assemblage because of their rarity. For each assemblage, the estimated total number of taxa (S_{est}) and total number of taxa shared with other assemblages ($S_{12(est)}$) were estimated using the *Chao1* and *Chao1-shared* estimators (Chao *et al*., 2006), enabling calculation of the estimated number of unique taxa ($U_{est} = S_{est} - S_{12(est)}$). Estimates of total taxon richness and shared taxa were carried out using the program SPADE (Chao and Shen, 2010). All other analyses were carried out using the PRIMER V6 software (Clarke and Gorley, 2006) with PERMANOVA+ v1 add-on (Anderson *et al*., 2008), except for the diversity profiles, which were constructed using PAST v2.17 (Hammer *et al*., 2001).

3.3 Results

A total of 39,993 individuals belonging to 333 macrofaunal taxa were recorded in all. The most common groups were the Mollusca (135 taxa), Crustacea (93 taxa) and Polychaeta (77 taxa). Spirorbinae spp. accounted for 45% of all individuals and 88% of the polychaetes, with nearly half of the spirorbids being recorded from a single site (Marsaxlokk). Polychaeta was the most abundant faunal group overall, but non-spirorbid polychaetes comprised only 6% of the total fauna. Apart from Spirorbinae, Crustacea and Mollusca were the most abundant groups (Figure 3.2). Other faunal groups included the Echinodermata, Cnidaria, Sipuncula, Nemertea, Tunicata and Actinopterygii which together accounted for only 1.5% of the total abundance.

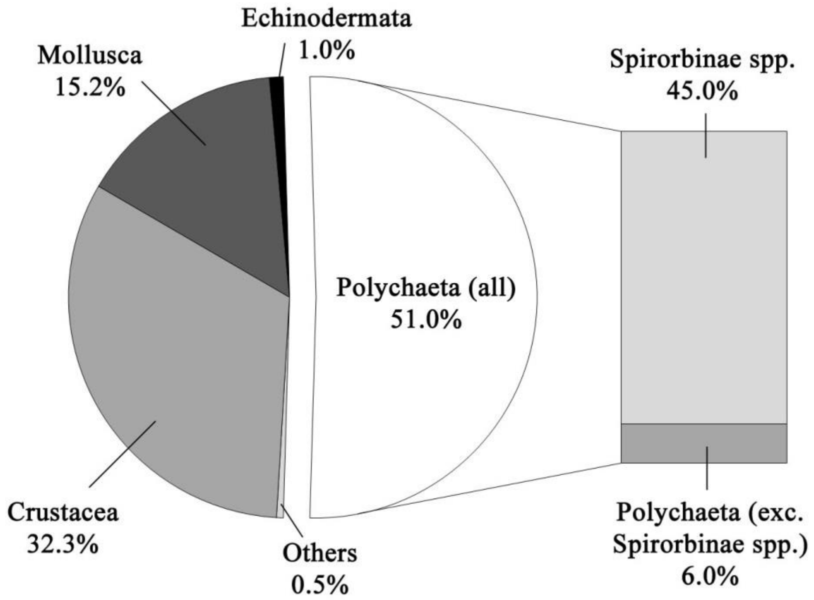

Figure 3.2 Percentage abundance of major faunal groups recorded from infralittoral pebble beds at 19 sites in the Maltese Islands.

PERMANOVA indicated that the species composition and abundance varied significantly between the four pebble-bed categories, with pair-wise tests showing that all four bed types differed from one another except in the case of the seagrass and creek pebble-bed types (Table 3.2). This result was corroborated by the outcome of the CLUSTER analysis, where three main clusters can be observed (Figure 3.3): cluster A represents the shallow sites, cluster B consists of sites that were *a priori* classified into the seagrass and creek categories, which can therefore be considered to belong to a single ‘seagrass/creek’ assemblage, while cluster C represents the harbour sites; SIMPROF tests failed to reject the null hypothesis of ‘no structure’ within any of the three clusters. As evidenced by the nMDS ordination plot (Figure 3.4), the seagrass/creek assemblage is somewhat intermediate between the shallow and harbour ones.

Table 3.2 Permutational multivariate Analysis of Variance based on the Bray-Curtis similarity measure for square-root transformed abundance data.

(a) Global test

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P (perm)**	**Unique perms**
Bed type	3	69759	23253	4.318	**0.001**	998
Site(Bed type)	15	80770	5385	5.809	**0.001**	998
Residual	57	52836	927			
Total	75	2.0×10^5				

(b) Pair-wise comparisons of different pebble-bed types

Groups	**t**	**P (perm)**	**Unique perms**
Shallow, Creek	1.316	**0.026**	126
Shallow, Seagrass	1.775	**0.013**	126
Shallow, Harbour	2.953	**0.003**	422
Creek, Seagrass	1.222	0.105	35
Creek, Harbour	2.435	**0.007**	209
Seagrass, Harbour	2.141	**0.007**	209

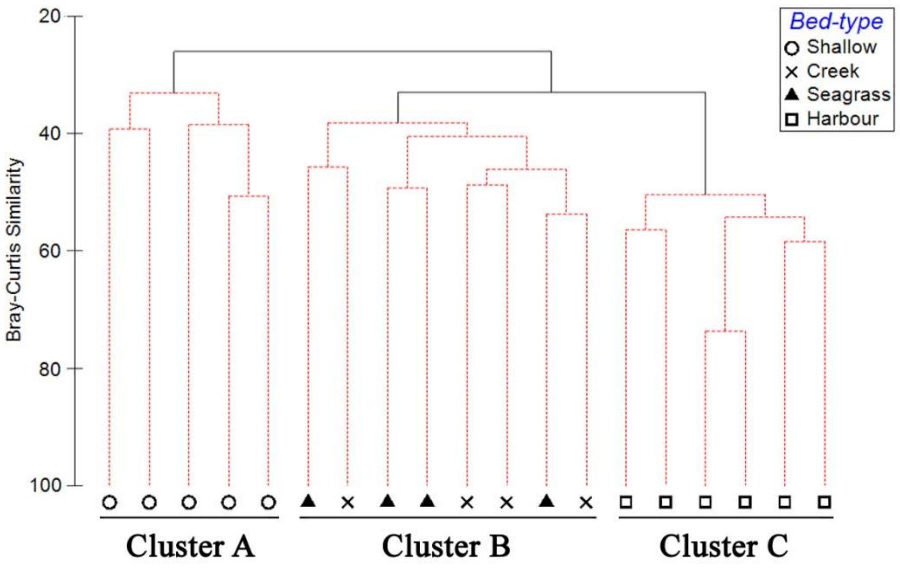

Figure 3.3 Dendrogram resulting from group-average hierarchical cluster analysis, based on Bray-Curtis resemblances produced using square-root transformed abundance data; sites which were not considered to be statistically different by the SIMPROF routine are shown connected by dotted red lines.

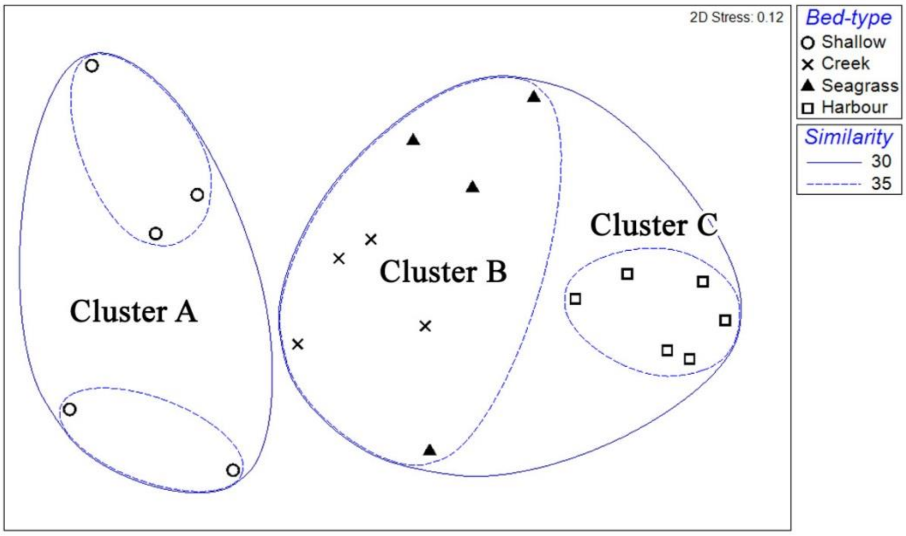

Figure 3.4 Non-metric multi-dimensional scaling ordination plot of the 19 sites, based on Bray-Curtis resemblances produced using square-root transformed abundance data. Dashed blue lines indicate the site groupings as identified by CLUSTER analysis (Figure 3.3).

The diversity profiles of the three clusters (Figure 3.5) did not intersect and could thus be meaningfully compared, yielding a diversity ordering of clusters of C > B > A. For mean diversity of order 0 ($^{0}\overline{D} \equiv \overline{S}_{\text{obs}}$, the observed taxon richness) and order 1 (i.e. Hill's diversity $^{1}\overline{D}$) the difference between clusters was statistically significant, mainly due to cluster A having lower values than the other two clusters (Table 3.3). A similar result was obtained for the mean estimated taxon richness ($\overline{S}_{\text{est}}$), but no significant differences were recorded in the case of mean total abundance ($\overline{TA}$), evenness measures ($^{1}\overline{D}/\overline{S}_{\text{obs}}$ and $\overline{J'}$) or taxonomic distinctness ($\overline{\Delta^{*}}$). On the other hand, values for average variation in taxonomic distinctness ($\overline{\Lambda^{+}}$) differed significantly among all three clusters, while cluster A had a significantly lower mean taxonomic diversity ($\overline{\Delta}$) than cluster C.

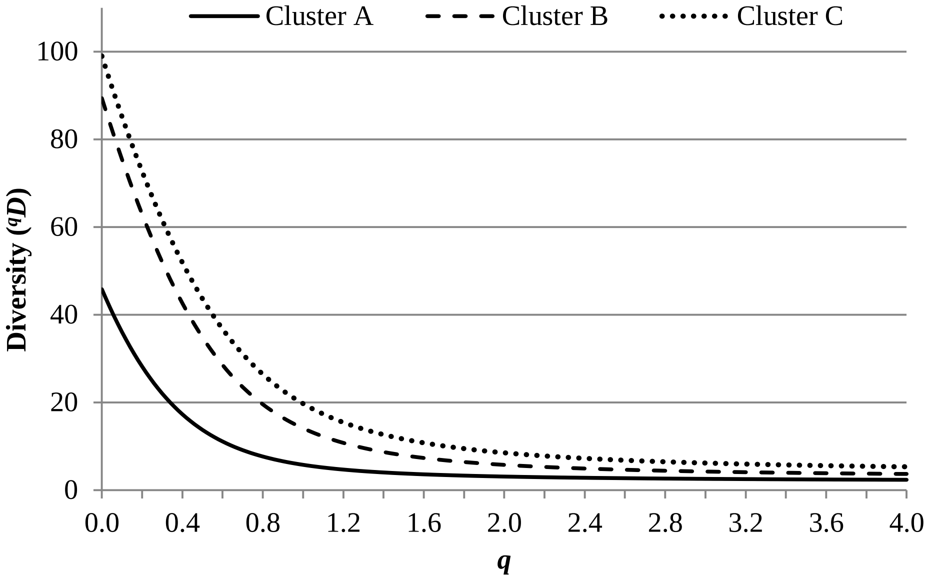

Figure 3.5 Mean diversity profiles for each of the three clusters shown in Figure 3.3. Each profile consists of a plot of Hill numbers (^qD) for values of q ranging from 0 to 4; as q increases, more weight is given to common species in the analysis.

Overall, a total of 123 different taxa were recorded from cluster A, compared to 260 from cluster B and 217 from cluster C. Estimated total taxon richness values (S_{est}) suggested that clusters B and C contained over 300 species each, of which 80–100 were unique to that particular cluster (U_{est}). In contrast, cluster A contained about half the number of taxa with an estimated total of 150 taxa, only 24 of which were unique to the cluster. However, the three clusters had unequal sampling areas, since cluster A contained only 5 sites, compared to 8 sites in cluster B and 6 sites in cluster C. To ensure that these results were not artefacts of unequal sampling effort, the analyses for clusters B and C were repeated using 5 sites per cluster, specifically choosing those sites which had the lowest taxon richness. Estimates obtained for cluster B were lower (S_{est} = 255; U_{est} = 49) than when using all 8 sites, while no substantial differences in S_{est} or U_{est} were evident for cluster C. Overall, cluster A still had the lowest estimated total taxon richness and number of unique taxa.

Table 3.3 Mean (± SD) values for various univariate diversity indices (see Methods for details), based on a sampling area of 0.4 m^2 per site, together with the actual and estimated number of total, shared and unique taxa for each of the three clusters shown in Figure 3.3.

	Mean per Site (± SD)			**PERMANOVA**		**Pair-wise Tests**		
	Cluster A	**Cluster B**	**Cluster C**	**Pseudo-F**	**P (perm)**	**A vs B**	**A vs C**	**B vs C**
$\overline{S}_{obs}$	45.8 ± 10.4	89.4 ± 22.5	99.0 ± 13.5	14.27	**0.001**	**0.002**	**0.003**	0.369
$\overline{S}_{est}$	72.2 ± 9.1	137.1 ± 29.4	142.1 ± 19.0	16.31	**0.001**	**0.001**	**0.004**	0.718
$\overline{TA}$	3089 ± 3554	1828 ± 1333	1654 ± 647	0.82	0.506	0.458	0.460	0.796
${}^{1}\overline{D}$	5.78 ± 2.79	14.11 ± 7.93	19.73 ± 9.91	4.43	**0.022**	0.052	**0.004**	0.296
${}^{1}\overline{D}/\overline{S}_{obs}$	0.12 ± 0.05	0.16 ± 0.08	0.2 ± 0.10	1.30	0.311	0.388	0.126	0.397
$\overline{J}'$	0.41 ± 0.20	0.56 ± 0.13	0.63 ± 0.09	3.39	0.051	0.152	0.002	0.256
$\overline{\Delta}$	50.7 ± 24.8	66.7 ± 12.9	75.2 ± 7.8	3.40	**0.039**	0.133	**0.005**	0.175
$\overline{\Delta^*}$	88.8 ± 8.3	88.9 ± 3.4	88.0 ± 2.7	0.06	0.932	0.979	0.891	0.599
$\overline{\Lambda^+}$	363.4 ± 35.0	317.9 ± 25.9	280.4 ± 16.7	13.68	**0.001**	**0.015**	**0.002**	**0.014**

	Total per Cluster		
	Cluster A	**Cluster B**	**Cluster C**
S_{obs}	123	260	217
S_{est}	150	328	307
$S_{12(obs)}$	105	185	167
$S_{12(est)}$	126	229	226
U_{obs}	18	75	50
U_{est}	24	99	81

The reduction in taxon richness at the shallow sites occurred in all major faunal groups (Figure 3.6) but especially in the case of molluscs and polychaetes; reductions in the number of decapod and amphipod crustacean taxa were partially offset by a slight increase in isopod richness. In terms of abundance, all the clusters contained similar quantities of Crustacea. A higher abundance of molluscs and echinoderms was recorded from harbour sites. The much lower polychaete abundance at these sites was entirely due to lower numbers of Spirorbinae; abundance of non-spirorbid polychaetes was similar in clusters B and C (143–150 ind./0.4m^2), and over twice that recorded from cluster A (65 ind./0.4m^2).

The results of SIMPER analysis are summarised in Table 3.4; only the top 10 contributing taxa are shown for each category. The number of taxa contributing up to 90% of the within-cluster similarity was only 23 for cluster A, compared to 58 for cluster B and 61 for cluster C. Some species (e.g. *Leptochelia savignyi*) contributed to the within-cluster similarity of all three clusters. Relatively high values for average dissimilarity between clusters were recorded, with the minimum dissimilarity being 67.1% (between clusters B and C).

Spirorbinae spp. was the taxon contributing most to dissimilarity between all cluster pairs, but did not have an overriding contributory influence to the observed dissimilarity (<11% in all cases); the number of taxa contributing to 90% dissimilarity between clusters ranged from 135 (cluster A vs C) to 171 (cluster B vs C). Two species of interest not included in Table 3.4 are the Maltese top-shell *Gibbula nivosa* and the date mussel *Lithophaga lithophaga*, which are both protected under the HD. Both were present at the harbour sites (cluster C), while *G. nivosa* was also recorded from the seagrass/creek bed type (cluster B).

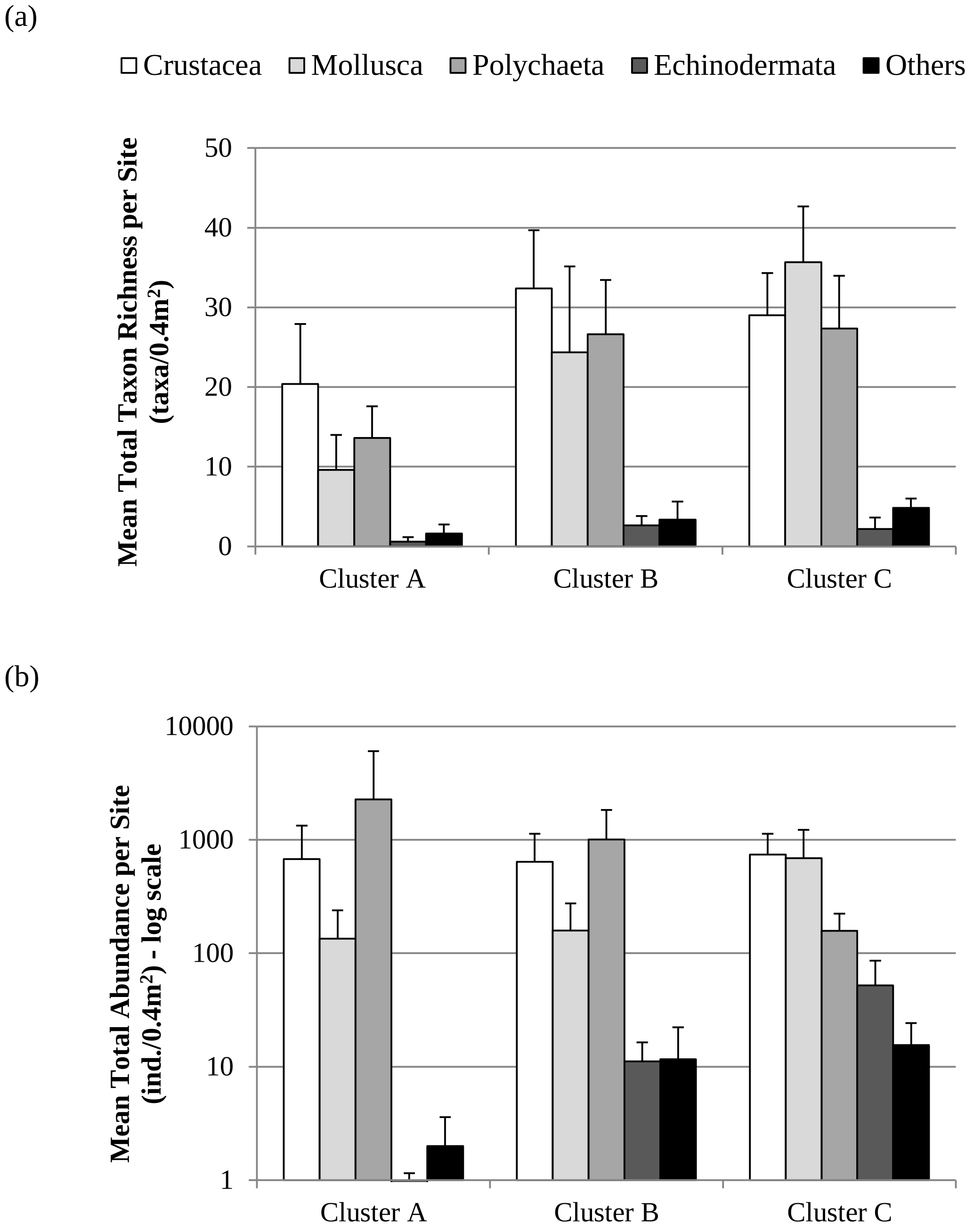

Figure 3.6 Mean (+ SD) values per site of (a) total taxon richness and (b) total abundance of the major faunal groups recorded during the present study; separate values are given for each of the three clusters shown in Figure 3.3.

Table 3.4 Output from SIMPER analysis, identifying main taxa contributing to (a) the within-cluster similarity for each of the three clusters shown in Figure 3.3, and to (b) the dissimilarities between cluster pairs; only the top 10 contributing taxa are shown for each category.

(a) Similarities

	Species	**Av.Abund**	**Av.Sim**	**Contrib%**
Cluster A (Shallow) *Average similarity: 36.55*	Spirorbinae spp.	17.51	9.27	25.37
	Melita hergensis	6.18	5.42	14.82
	Gibbula varia	3.87	4.29	11.73
	Leptochelia savignyi	4.04	3.05	8.33
	Gammarella fucicola	5.24	2.45	6.70
	Clibanarius erythropus	2.21	1.37	3.74
	Xantho pilipes	1.19	1.00	2.73
	Opheliidae sp. B	0.91	0.85	2.33
	Microdeutopus spp.	1.38	0.66	1.81
	Ampithoe ramondi	1.31	0.59	1.61
Cluster B (Segrass/Creek) *Average similarity: 41.55*	Spirorbinae spp.	12.76	6.87	16.53
	Leptochelia savignyi	5.56	3.04	7.33
	Maera grossimana	3.53	1.97	4.75
	Microdeutopus spp.	3.30	1.77	4.27
	Gammarella fucicola	2.89	1.50	3.60
	Nereis rava	1.78	1.24	2.99
	Ischnochiton rissoi	2.05	1.24	2.99
	Ampithoe ramondi	2.02	1.19	2.86
	Xantho pilipes	1.92	1.04	2.49
	Serpulidae spp.	1.99	0.96	2.30
Cluster C (Harbour) *Average similarity: 53.89*	*Pisidia bluteli*	7.48	4.29	7.95
	Alvania mamillata	8.32	4.00	7.42
	Leptochelia savignyi	6.73	2.99	5.55
	Athanas nitescens	3.24	2.34	4.33
	Ischnochiton rissoi	3.60	2.23	4.13
	Amphipholis squamata	3.33	1.98	3.67
	Calcinus tubularis	2.50	1.71	3.17
	Paraonidae sp. A	2.68	1.67	3.10
	Bittium reticulatum	3.07	1.58	2.93
	Cyathura sp.	1.85	1.30	2.40

Table 3.4 (continued)

(b) Dissimilarities

	Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Contrib%**
Cluster A vs Cluster B *Average disimilarity: 68.50*		**Cluster A**	**Cluster B**		
	Spirorbinae spp.	17.51	12.76	7.03	10.26
	Melita hergensis	6.18	1.6	2.78	4.05
	Gammarella fucicola	5.24	2.89	2.10	3.06
	Maera grossimana	0.20	3.53	1.82	2.65
	Leptochelia savignyi	4.04	5.56	1.81	2.64
	Gibbula varia	3.87	1.40	1.61	2.35
	Microdeutopus spp.	1.38	3.30	1.16	1.70
	Clibanarius erythropus	2.21	0.78	1.11	1.62
	Serpulidae spp.	0.10	1.99	1.06	1.55
	Bittium latreillii	0.10	2.06	1.00	1.47
Cluster A vs Cluster C *Average disimilarity: 81.39*		**Cluster A**	**Cluster C**		
	Spirorbinae spp.	17.51	1.06	7.92	9.73
	Alvania mamillata	0.32	8.32	3.91	4.80
	Pisidia bluteli	0.54	7.48	3.38	4.15
	Melita hergensis	6.18	0.26	2.92	3.59
	Leptochelia savignyi	4.04	6.73	2.20	2.70
	Gammarella fucicola	5.24	1.39	1.97	2.42
	Gibbula varia	3.87	0.31	1.78	2.19
	Ischnochiton rissoi	0.36	3.60	1.58	1.94
	Amphipholis squamata	0.20	3.33	1.54	1.90
	Athanas nitescens	0.30	3.24	1.44	1.77
Cluster B vs Cluster C *Average disimilarity: 67.07*		**Cluster B**	**Cluster C**		
	Spirorbinae spp.	12.76	1.06	4.75	7.08
	Alvania mamillata	0.64	8.32	3.24	4.83
	Pisidia bluteli	0.44	7.48	2.98	4.45
	Leptochelia savignyi	5.56	6.73	1.85	2.76
	Maera grossimana	3.53	1.54	1.07	1.60
	Bittium reticulatum	0.58	3.07	1.04	1.55
	Microdeutopus spp.	3.30	0.68	1.01	1.51
	Clanculus jussieui	0.00	2.26	0.95	1.42
	Alvania lineata	0.26	2.39	0.87	1.30
	Paraonidae sp. A	0.64	2.68	0.86	1.28

3.4 Discussion

A total sampling area of 7.6 m^2 of pebble habitat yielded 333 different macrofaunal taxa and just under 40,000 individuals (22,000 excluding the highly abundant Spirorbinae). These numbers contradict previous assertions that Mediterranean pebble beds are species poor and denuded habitats (e.g. Bellan-Santini, 1985). However, the high taxon richness recorded in the present study may partly be due to inclusion of different assemblage types, in contrast with previous descriptions of the Mediterranean biocoenosis of infralittoral pebbles, which were restricted to those beds found in very shallow waters. In the Maltese Islands, three different pebble-bed habitats could be distinguished based on their biotic composition: shallow beds, beds occurring within creeks or amongst seagrasses, and beds found in sheltered harbour sites. This classification is very similar to that obtained when analysing environmental data (see Chapter 2).

The shallow pebble-bed assemblages were more or less equivalent to the biocoenosis of infralittoral pebbles as described in habitat manuals (Pérès and Picard, 1964; Pérès, 1967; Bellan-Santini *et al.*, 1994), including characteristic species such as *Melita hergensis*, *Gibbula* spp. and *Xantho pilipes*. On the other hand, the amphipod *Parhyale aquilina* was only recorded from two of the five shallow sites, while the clingfish *Gouania willdenowi* was not present in any of the samples. The sampling techniques used may have under recorded these fish, but these techniques successfully collected a few individuals of another clingfish, *Lepadogaster* sp., as well as a number of gobiid fish. It therefore seems unlikely that all the *G. willdenowi* individuals would have evaded capture had this species been present at a high abundance. Thus, it appears that a "facies with *Gouania willdenowi*" (UNEP-MAP-RAC/SPA, 2006a) did not occur at the sampled locations.

Two individuals of *Lepadogaster* sp., which are normally found in areas with larger pebbles according to Pérès and Picard (1964) and Pérès (1967), were recorded from Qawra. Other species not listed in the available habitat descriptions but which were typical of the Maltese shallow pebble-bed type included the amphipod *Gammarella fucicola* and the hermit crab *Clibanarius erythropus*. Spirorbinae were found at an exceptionally high abundance at Marsaxlokk, and were also abundant at three of the other four sites; these generally occurred as dense aggregations on a few of the larger pebbles, possibly because here they are less susceptible to physical disturbance.

Accumulations of pebbles within creeks or in inter-matte regions of reticulate *Posidonia oceanica* beds at depths reaching 12 m constituted the second pebble-bed habitat recorded from the Maltese Islands. Therefore, seagrass and creek pebble-bed types had similar faunal composition despite being bordered by different adjacent habitats, suggesting that they contain a particular assemblage type associated with the pebble bed itself; this is corroborated by the fact that these bed types also had similar physical characteristics (see Chapter 2). However, the extent to which the pebble-bed assemblage contains unique species or shares fauna with adjacent habitats can only be ascertained by sampling of the other habitats.

The seagrass/creek assemblage was somewhat intermediate between those found in shallower sites and those found in harbour environments, with many of the more common species being present in one or both of these other pebble-bed types. It was characterised by a higher abundance of the amphipods *Maera grossimana* and *Microdeutopus* spp. and serpulid polychaetes. Spirorbinae were also highly abundant at most sites except Gnejna, with similar abundances to those that were recorded from the shallow pebble beds (excluding Marsaxlokk).

The third pebble-bed type recorded from the Maltese Islands occurred in Marsamxett Harbour. These beds were characterised by a high percentage cover of encrusting coralline algae, and a higher content of fine particles belonging to the sand and silt fractions was present within the bed, probably due to the more sheltered conditions (Chapter 2). This assemblage differed from the others due to the higher abundance of the porcelain crab *Pisidia bluteli*, of the gastropods *Alvania* spp. and *Bittium reticulatum*, and of the ophiuroid *Amphipholis squamata*. On the other hand, comparatively low numbers of Spirorbinae were present, possibly due to the competition for space with encrusting algae. *Pisidia bluteli* is listed by Pérès and Picard (1964) and Pérès (1967) as occurring in crevices within infralittoral hard substrata and invading pebble beds when these consist of larger pebbles. Harbour sites, however, had slightly smaller pebbles than elsewhere, although these are likely to be subjected to less physical disturbance given their sheltered location.

If similar differences between pebble-bed types are noted elsewhere in the Mediterranean, then it would be appropriate to revise the Mediterranean benthic habitats classification scheme (UNEP-MAP-RAC/SPA, 2006a) to reflect the existence of these different pebble assemblages. Category III.4 “Stones and pebbles” could be retained, but sub-category III.4.1 “Biocoenosis of infralittoral pebbles” would need to be replaced by a “Biocoenosis of infralittoral pebbles in exposed shallow waters”, while another two sub-categories could be added to account for the seagrass/creek and harbour pebble-bed assemblages described above. This would bring the classification scheme for infralittoral pebbles in line with those for other mobile substrata, each of which contains more than one biocoenosis.

A more extensive review appears to be necessary when it comes to the EUNIS habitat classification scheme. Within the Level 4 habitat code A5.13 “Infralittoral coarse sediment”, the scheme currently includes eighteen different habitat sub-types, six of which refer to stone or pebble assemblages, with the remainder being coarse sand and gravel biotopes. The Mediterranean pebble habitat is listed simply as A5.139 “Facies with *Gouania willdenowi*”, but for other soft substrata in the EUNIS scheme it is the biocoenoses which are listed at this level (Level 5), not their respective facies or associations. Thus, with the current schemes this would more appropriately be listed as “Mediterranean biocoenosis of infralittoral pebbles”, with the *G. willdenowi* facies being present as a sub-type at Level 6. Any changes to the UNEP-MAP-RAC/SPA scheme as suggested above could then be reflected in the EUNIS scheme by including the three pebble-bed biocoenoses at Level 5.

In addition, some of the biotopes derived from different classification schemes and incorporated into EUNIS may be functionally equivalent. It is beyond the scope of the present work to undertake a comparative analysis of the different biotopes but consider, for instance, the similarities between the British “Sparse fauna on highly mobile sublittoral shingle (EUNIS A5.131)”, the “Pontic scoured sublittoral cobbles and pebbles with sparse crustaceans (EUNIS A5.13H)”, and the Mediterranean biocoenosis of infralittoral pebbles in exposed shallow waters. Galparsoro *et al*. (2012) have recently suggested including substratum stability as a grouping criterion for soft substrata, which would aid in creating a more coherent classification scheme for these habitats.

The pebble-bed assemblages described here can be meaningfully used in benthic mapping and its applications in environmental management and conservation since they were derived on the basis of biological data, and are hence useful as surrogates for the diversity they contain. Given that the shallow bed types contained an estimated 150 macrofaunal taxa, they cannot be considered to be impoverished. However, most of the recorded taxa were shared with at least one of the other pebble assemblages and only a low number of unique taxa were present. In addition, the faunal composition and richness of shallow mobile pebbles may vary seasonally as a result of disturbance from increased wave action in winter (Connor *et al*., 2004). A seasonal investigation is necessary to determine whether any substantial reduction in diversity occurs as a result of physical disturbance (see Chapter 4).

On the other hand, pebble beds found in deeper waters were more diverse (mean values of taxon richness and Hill diversity were twice as high) than those occurring in shallow locations. Both the seagrass/creek and harbour bed types were estimated to include over 300 taxa, with a substantial proportion of unique species. The percentage abundance of faunal groups recorded from these bed types was similar to those reported by Linnane *et al*. (2001) from cobble beds found at similar depths (7–17 m) in Norway, UK and Italy, where crustaceans were the dominant motile fauna followed by molluscs and polychaetes; in contrast, molluscs were dominant at an Irish cobble site (Linnane *et al*., 2001, 2003). These authors provide estimates for the number of decapod species, ranging between 15–32 species (with sampling areas of 7–20 m^2), similar to the 17 decapod species recorded by Robinson and Tully (2000a) from a different location in Ireland (depth 6–12 m; sampled area 5 m^2), and to the 20–24 decapod species recorded from the Maltese pebble-bed types found at similar depths. Notwithstanding the wide geographical separation of these locations, a number of decapod species were common to all sites, and are therefore seemingly associated with pebble/cobble habitats.

Only Linnane *et al*. (2003) provide details for other taxa besides decapods, which included more bivalve species but less gastropod and amphipod ones than those recorded from the seagrass/creek and harbour bed types in the present study. Overall, it is clear that these pebble-bed assemblages are quite species rich. This may be due to an intermediate disturbance regime (Osman, 1977), or to the physically complex nature of the substratum (Robinson & Tully 2000a, 2000b) with interstitial spaces of various dimensions providing refugia from predators for animals of different sizes (Linnane *et al*., 2003).

In this sense, pebble beds may be similar to the highly diverse rhodolith beds (e.g. Steller *et al*., 2003), which are considered to be priority habitats (UNEP-MAP-RAC/SPA, 2006b) having a high conservation value (Bellan-Santini *et al*., 1994; Barbera *et al*., 2003). Although rhodolith assemblages are mainly found in the lower infralittoral and circalittoral in the Mediterranean, they have also been documented from the upper infralittoral, including within *Posidonia oceanica* inter-matte canals (Relini and Giaccone, 2009), while rhodoliths were present within the pebble beds found in Marsamxett Harbour during the present study.

These results indicate that pebble beds can be highly diverse and hence of potential conservation value, especially in the case of seagrass/creek and harbour pebble-bed types. The presence of unique or rare species within pebble beds would further enhance their conservation value, but this can only be ascertained through detailed comparative studies of the species assemblages found in different habitats. On the other hand, pebbles beds may still represent unique assemblages, even if none of their component species occur solely in pebble beds. Furthermore, pebble beds have a low total coverage due to their occurrence as distinct patches of relatively small area (Chapter 2), and are therefore rare habitats.

Taken together, these observations suggest that the occurrence of pebble-bed habitats should be taken into consideration when siting MPAs. In the case of the Maltese Islands, live populations of the critically endangered endemic top-shell *Gibbula nivosa* have only been recorded from cobble and pebble habitats since the rediscovery of this species in 2006 (Evans *et al*., 2010, 2011; this study). Since *G. nivosa* is included in Annex II of the HD (species whose conservation requires designation of protected areas), infralittoral pebble beds should actually be considered as priority habitats when formulating conservation management plans and designating MPA boundaries in Maltese coastal waters.

Chapter 4:

Seasonal variation in the motile macrofaunal assemblage associated with infralittoral pebble beds in the Maltese Islands

Chapter 4

Seasonal variation in the motile macrofaunal assemblage associated with infralittoral pebble beds in the Maltese Islands

Abstract

Past studies have highlighted the role of disturbance in driving seasonal changes in the composition of sessile assemblages found in infralittoral pebble and cobble habitats. In contrast, the effects of disturbance on the motile macrofauna have not been well-documented. In the present study, quantitative samples of the motile macrofauna occurring in five shallow-water infralittoral pebble beds in the Maltese Islands were collected on a biannual basis over a two-year period (September 2011 – April 2013). A significant reduction in total abundance was recorded following winter disturbance in both years. This brought a concomitant decrease in the number of taxa per sampled area, but not in the overall diversity of the assemblage, nor in the taxon richness when standardised to account for the differences in abundance. This seasonal pattern was mainly due to changes in richness and abundance of molluscs and crustaceans; no seasonal trend was discernible in the case of polychaetes. These findings suggest that physical disturbance is an important driver of seasonal fluctuations in pebble-bed motile macrofaunal assemblages; winter reductions in richness and abundance caused by disturbance are counterbalanced by repopulation of these habitats during the calm summer months.

4.1 Introduction

Understanding the processes that determine spatio-temporal variation in species distribution patterns and the structure of communities is a major focus of ecological research. Following Dayton's (1971) seminal work, disturbance has been recognised as a primary mechanism structuring a variety of marine benthic assemblages (reviewed by Sousa, 2001). Disturbance is generally defined as the removal of biomass via damage or mortality (Grime, 1977). Various agents of disturbance have been identified, including both physical (e.g. storm waves or currents) and biological (e.g. bioturbation) causes (Sousa, 2001). Their ecological effects depend on the type, frequency and intensity of the disturbance regime (Sousa, 1984). However, the frequencies and intensities of disturbing forces vary temporally and often have a strong seasonal component, particularly in the case of physical agents such as storm waves (see Sousa, 2001 and references therein). Thus, temporal patterns of physical disturbance may play an important role in determining seasonal variation in the structure of marine benthic assemblages.

Intertidal boulder fields and sublittoral pebble or cobble beds are marine habitats with distinctive physical characteristics (Scheibling *et al*., 2009a). Because of the relatively mobile nature of pebbles, cobbles and small boulders, strong waves and currents can cause overturn or displacement of the substrate, leading to abrasion, crushing or detachment of the attached sessile biota. As a result, physical disturbance is the primary force influencing species diversity and survival in these habitats (Osman, 1977; Lieberman *et al*., 1979, 1984; Sousa, 1979; Littler and Littler, 1984; Davis and Wilce, 1987a; McGuinness, 1987a, 1987b; Scheibling *et al*., 2009a). The disturbance frequency does not depend solely on the hydrodynamic regime, but also on the stability of the substratum itself: the rate of disturbance decreases with increasing particle size, since larger particles have a greater moment of inertia (Osman, 1977; Lieberman *et al*., 1979; Sousa, 1979).

Studies carried out on subtidal cobble habitats have focused on differences in sessile biota found on cobbles of different sizes (Osman, 1977; Lieberman *et al*., 1979; Davis and Wilce, 1987a). These studies indicated that medium-sized cobbles had the highest species diversity due to intermediate levels of disturbance, thus lending support to Connell's (1978) intermediate disturbance hypothesis. Such studies ultimately depend on temporal variation in the physical forces mediating disturbance, with forces strong enough to overturn the largest cobbles occurring only during seasonal storm events (e.g. Lieberman *et al*., 1979). The effect of temporal trends in disturbance *per se* was not investigated by Davis and Wilce (1987a). On the other hand, Lieberman *et al*. (1979) noted a marked seasonal variation in macroalgal assemblages, with most algae being destroyed as a result of cobble tumbling during the stormy season. Similarly, in the case of epifauna, Osman (1977) observed a general reduction in growth rates and loss of several species during winter, with seasonal disturbance having the greatest effect on the epifaunal composition of cobbles occurring in very shallow water (0.5 m depth).

In contrast to sessile species, the role of disturbance in structuring assemblages of motile macrofauna has been little studied. According to Sousa (1984, 2001), this may simply be due to the fact that responses of motile organisms are less easy to observe and quantify. However, there are also fundamental differences in the way sessile and motile organisms respond to agents of disturbance, since motile animals can behaviourally avoid potentially lethal environmental stresses. On the other hand, if their response is not fast enough relative to the development of the disturbance conditions, physical forces such as storm waves or scour by cobbles can directly damage or kill appreciable numbers of motile animals (Sousa, 2001). Such a situation may occur more readily with slow moving animals such as limpets, chitons and sea urchins (Ebeling *et al*., 1985; Dayton *et al*., 1989) but has also been documented in coral reef fish (Lassig, 1983). By altering the composition and structure of sessile assemblages, physical disturbance can also have an indirect effect on populations of motile animals, which depend on their sessile counterparts for food and shelter (Dean and Connell, 1987a, 1987b, 1987c).

Dean and Connell (1987a) noted temporal variation in the motile invertebrate assemblage structure associated with intertidal boulders as a result of increased disturbance in winter. Reductions in richness, abundance and diversity were especially pronounced in the case of smaller boulders. This suggests that habitats that consist predominantly of smaller particles such as pebble beds may be particularly prone to seasonal changes in assemblage structure of motile fauna. According to descriptions in habitat classification manuals, shallow-water pebble beds occur in wave exposed environments and typically lack any conspicuous epibiota, reflecting the unstable nature of the substratum (Pérès and Picard, 1964; Connor *et al*., 2004).

However, it appears that no studies on the effects of disturbance on the motile fauna in pebble habitats have been published in the mainstream literature, so no quantitative assessment of seasonal changes in the pebble-bed motile macrofaunal assemblage structure is available. Furthermore, contrasting information is given in different habitat manuals. In describing the British biotopes, Connor *et al*. (2004) suggest that the faunal composition of pebble habitats is likely to be highly variable as a result of seasonal changes in wave and tidal energy. On the other hand, Pérès and Picard (1964) state that motile animals found among Mediterranean infralittoral pebbles can escape to more sheltered locations during stormy weather and subsequently return to the pebble habitat as soon as the sea becomes calm. In this case, one would expect to record only minimal seasonal variation in faunal composition, provided that sampling is always undertaken in calm conditions.

In the Maltese Islands, wind-generated waves are the primary agents of physical disturbance in infralittoral habitats. Here, the lowest mean wind speeds occur between July and September, so the intensity of disturbance is lowest during these summer months. In contrast, wind-generated hydrodynamic forces are strongest between January and March, given the increased frequency and intensity of gale force winds that occur during this period (Galdies, 2011). Biotic data collected from Maltese shallow-water pebble beds as part of a related study (see Chapter 3) indicated that these habitats may not be as impoverished as previously thought (e.g. Bellan-Santini, 1985). Since this study was based on sampling carried out in September, the number of species and their abundances may have been relatively high due to recent recruitment or immigration of adults from adjacent habitats during the calm summer months. In the absence of studies on the seasonal variation of pebble-bed assemblages, it is not clear whether these fauna persist throughout the year, or if significant reductions in species richness and abundance occur as a result of winter storms. The present study was therefore undertaken to investigate the effect of seasonal disturbance on the motile macrofauna associated with these shallow-water sublittoral pebble beds.

4.2 Material and methods

Previous surveys carried out along the low-lying coasts of the Maltese Islands identified five locations where substantial accumulations of pebbles occur in shallow waters (Figure 4.1). These sites are characterised by a bottom consisting of smooth pebbles covering an area of 25–50 m^2 at depths less than 2 m. Sampling was carried out at these sites on four separate occasions, twice during the post-summer months (September/October 2011 and 2012) and twice at the start of spring (March/April 2012 and 2013). The sampling dates were chosen at random but were subject to having calm weather conditions. During each sampling session, four replicate samples were collected from each of the five sites, for a total of 80 samples in all. In the field, sample position was selected randomly but no samples were collected towards the edges of the pebble beds to avoid edge effects (e.g. Todd and Turner, 1986).

Sampling was carried out using a 0.1 m^2 circular sampler (modified from Borg *et al.*, 2002) which was pushed into the substratum to delineate the sampling area and prevent escape of motile invertebrates. Cobbles and pebbles were hand-collected and transferred to a 0.5 mm mesh bag, while a small fine-mesh hand net (1 mm mesh size) was used to scoop the basal layer of finer granules. The samples were subsequently transported to the laboratory and preserved in 10% formaldehyde in seawater. They were later rinsed and sorted, separating the motile macrofauna (retained by a 0.5 mm sieve) into major taxonomic groups. The biota were identified to the lowest taxon possible and enumerated to construct a taxon-abundance matrix.

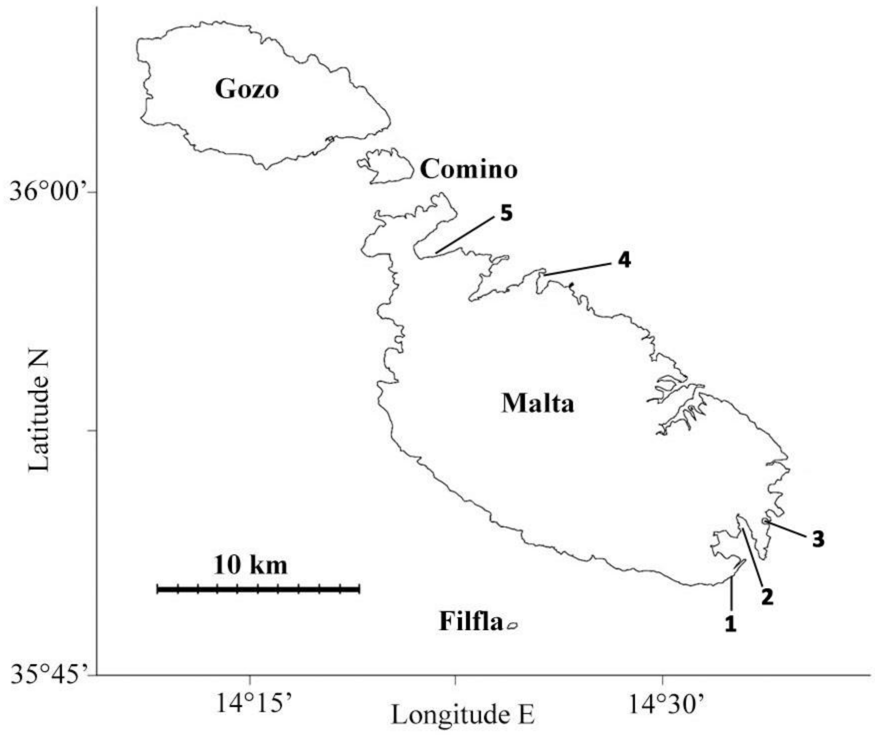

Figure 4.1 Map of the Maltese Islands indicating the position of the five sampling sites used in the present study.

Analyses of seasonal changes were made using both univariate and multivariate techniques. Values of the observed taxon richness (S_{obs}), total abundance (*TA*), Hill's diversity (^1D), evenness ($^1D/S_{obs}$) and relative evenness (Pielou J'), together with observed richness and abundance measures for each of the three major faunal groups (Mollusca, Crustacea, Polychaeta), were estimated for each replicate sample. Permutational Analysis of Variance (PERMANOVA; Anderson, 2001) was subsequently used to test for significant differences between seasons in terms of these parameters, based on Euclidean distance as the resemblance measure. Each parameter was tested individually, so the analyses were essentially univariate ones analogous to traditional ANOVA. However, in PERMANOVA the null distribution of the test statistic is produced by permutation, thus avoiding the usual assumptions of parametric tests (Anderson, 2001). The analyses consisted of a three-way mixed model having a fully-crossed and balanced design, with ‘Year’ (2 levels: Year 1 and 2) and ‘Season’ (2 levels: autumn and spring) as fixed factors and ‘Site’ as a random factor.

Since the number of taxa increases with the number of individuals encountered, the observed taxon richness is sensitive to sample size; a decrease in the number of taxa per area could be the result of having fewer individuals in the sample (Gotelli and Colwell, 2001). This can be a problem when evaluating the effects of disturbance on richness, because disturbance often reduces abundance (McCabe and Gotelli, 2000). Comparison of richness among samples that differ in the total number of individuals is often performed via rarefaction of larger samples to the size of the smallest one, or via extrapolation of species-accumulation curves of smaller samples. Colwell *et al*. (2012) recently developed a novel method that integrates these two techniques to produce a unified species-accumulation curve, standardised by sample size, together with estimates of confidence intervals for each point along the curve. This approach was used to compare the estimated taxon richness for the four sampling sessions. Data from all samples collected in a season were pooled to create the “abundance reference sample” used in the analysis (Colwell *et al*., 2012). The pooled data were also used to construct a cumulative ranked species-abundance curve for each season (k-dominance curves; Lambshead *et al*., 1983) in order to compare their dominance structure.

Multivariate analysis of seasonal changes in the taxon-abundance matrix was carried out via PERMANOVA, using the same design as for the univariate case. Non-metric multidimensional scaling (nMDS) was also performed to visualise the sample ordination pattern, based on site-averaged abundance data. These analyses were made using the Bray-Curtis similarity resemblance measure, calculated on square-root transformed data. The Bray-Curtis coefficient was chosen since it satisfies a number of biologically desirable criteria, providing an intuitive measure of ecological similarity (Clarke, 1993; Legendre and Legendre, 1998), but it tends to be unduly dominated by counts for highly-abundant taxa. A mild square-root transformation was therefore applied to down-weight the importance of such highly-abundant taxa in order to allow taxa of intermediate abundance to also contribute to the similarity calculations, while not providing too much weight to rarer taxa (Clarke and Green, 1988). The main characteristic and discriminating taxa for each season were then determined using Similarity Percentage analysis (SIMPER; Clarke, 1993). Univariate and multivariate analyses were carried out using the PRIMER V6 software (Clarke and Gorley, 2006) with PERMANOVA+ v1 add-on (Anderson *et al.*, 2008), while sample-size-based rarefaction and extrapolation curves were constructed using the iNEXT online software (Hsieh *et al.*, 2013).

4.3 Results

A total of 17,470 individuals belonging to 163 macrofaunal taxa were recorded across all sampling sessions. The most common groups overall were the Crustacea (60 taxa), Polychaeta (49 taxa) and Mollusca (43 taxa), with crustaceans being the most abundant group accounting for 78.8% of all individuals. The overall taxon richness varied seasonally, with 119 and 80 taxa recorded during autumn 2011 and 2012 respectively, compared to 66 taxa recorded in spring 2012 and 56 in spring 2013. These represent a reduction in the number of taxa of 30–45% in spring compared to the previous autumn. Similarly, total abundance decreased from 4,392 individuals in autumn 2011 to 1,801 individuals in spring 2012 (59% reduction), and from 10,362 individuals in autumn 2012 to just 915 individuals in spring 2013 (91% reduction).

Mean values per site of the univariate parameters are given in Table 4.1. When comparing the values obtained for the autumn sampling sessions with those of the subsequent spring sessions, a reduction in mean observed taxon richness and mean total abundance was evident for nearly all sites, although site to site differences were also apparent. The PERMANOVA analyses, which were used to test for a seasonal effect over and above any variability among sites, indicated that such an effect on mean taxon richness and abundance was in fact significant (Table 4.1). On the other hand, no apparent seasonal changes were evident for values of Hill Diversity or Pielou’s relative evenness, while absolute evenness measures recorded in spring were slightly higher than those recorded in autumn. The results of the PERMANOVA analyses indicated no significant seasonal effect for any of these three parameters.

Table 4.1 Mean (± SD) values for various univariate diversity indices (see Methods for details), based on a sampling area of 0.4 m^2 per site, together with a summary of the results of univariate PERMANOVA based on Euclidean distance as the resemblance measure; only results for the factor ‘Season’ are shown.

	Mean per Site (± SD)				**PERMANOVA**	
	Autumn 2011	**Spring 2012**	**Autumn 2012**	**Spring 2013**	**Pseudo-F**	**P (perm)**
All Fauna						
$\bar{S}_{\text{obs}}$	44.2 ± 10.1	26.0 ± 12.2	32.2 ± 13.7	19.8 ± 8.0	8.724	**0.040**
$\overline{TA}$	878.4 ± 710.0	360.2 ± 208.8	2072 ± 2746	183.0 ± 83.7	2.192	**0.035**
$^1\bar{D}$	8.45 ± 1.58	5.36 ± 2.15	6.32 ± 2.74	6.93 ± 3.06	2.061	0.221
$^1\bar{D}/\bar{S}_{\text{obs}}$	0.20 ± 0.06	0.27 ± 0.20	0.21 ± 0.08	0.35 ± 0.06	3.106	0.126
$\bar{J}'$	0.56 ± 0.06	0.54 ± 0.14	0.52 ± 0.10	0.63 ± 0.08	1.534	0.203
Crustacea						
$\bar{S}_{\text{obs}}$	20.4 ± 7.5	10.2 ± 3.4	13.0 ± 6.2	8.2 ± 3.1	8.979	**0.049**
$\overline{TA}$	676.8 ± 661.4	229.6 ± 169.9	1738 ± 2750	109.2 ± 72.9	1.699	0.065
Mollusca						
$\bar{S}_{\text{obs}}$	9.4 ± 4.0	2.2 ± 1.6	7.2 ± 3.8	3.0 ± 1.0	32.654	**0.008**
$\overline{TA}$	134.6 ± 104.9	13.8 ± 11.1	283.0 ± 148.4	26.8 ± 24.3	94.407	**0.001**
Polychaeta						
$\bar{S}_{\text{obs}}$	7.6 ± 3.9	11.0 ± 7.3	10.6 ± 5.1	7.6 ± 4.7	0.993	0.449
$\overline{TA}$	45.0 ± 53.4	97.6 ± 99.6	49.0 ± 36.1	45.0 ± 33.7	0.333	0.606

Mean total taxon richness and abundance per site for Crustacea and Mollusca follow a similar seasonal trend to that recorded when considering all fauna (Figure 4.2). In contrast, no reduction in spring polychaete abundance was discernible.

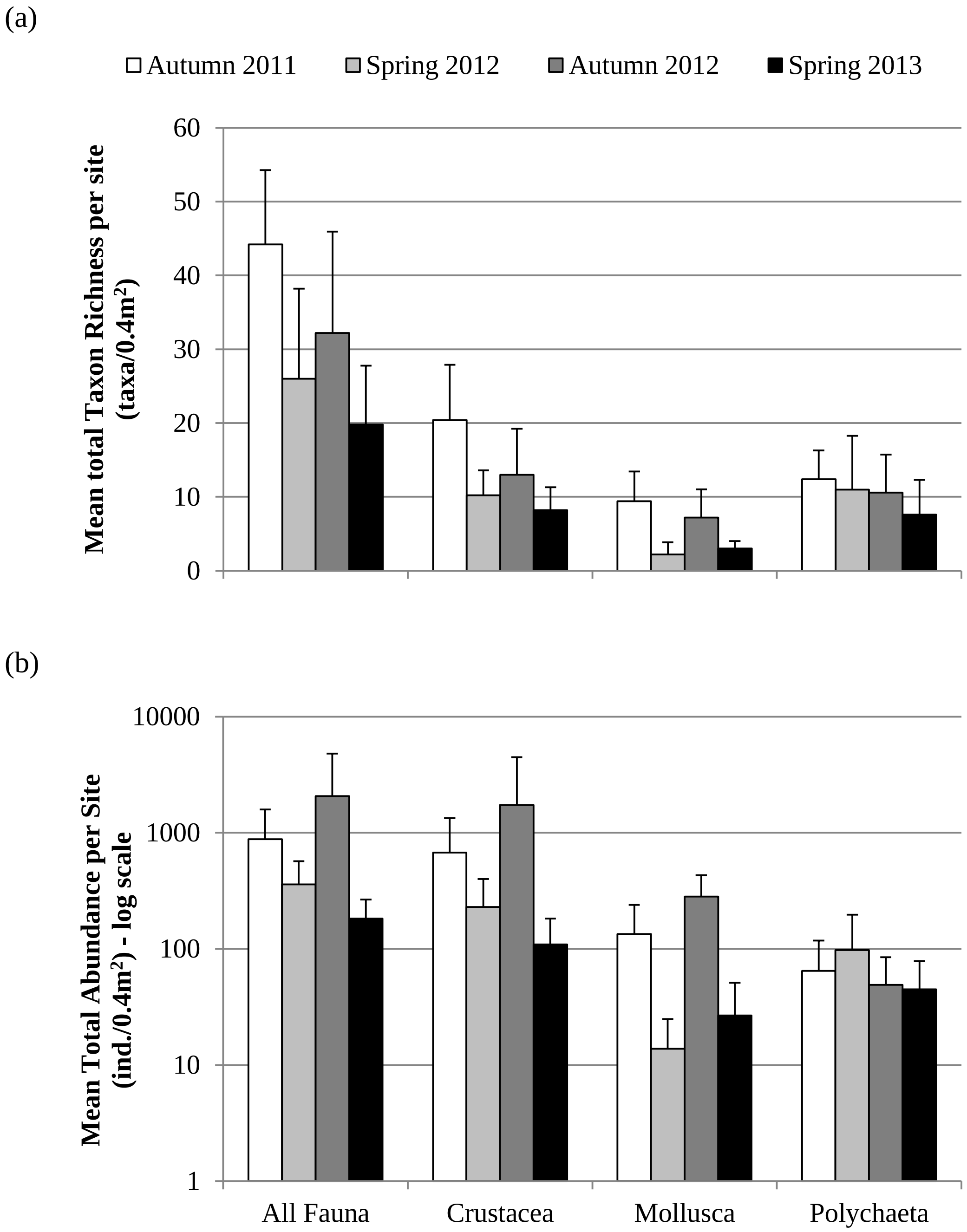

Figure 4.2 Mean (+ SD) values per site of (a) total taxon richness and (b) total abundance for each of the four sampling sessions, when considering all collected fauna, or each of the three major faunal groups recorded during the present study.

PERMANOVA indicated a significant seasonal effect for molluscan richness and abundance and for crustacean richness, but not for polychaete richness and abundance. Seasonal differences in crustacean abundance were also not significant, but the reported *p*-value was only slightly higher than the 0.05 significance level (Table 4.1). Overall, the greatest seasonal effect was seen for the Mollusca, with reductions of 58–77% in richness and 90% in abundance occurring from autumn to spring, compared to a 37–50% decrease in richness and 66–93% decrease in abundance for the Crustacea. No significant interaction for the term 'Year x Season' was recorded in any of the univariate analyses, showing that the recorded seasonal patterns were consistent across the two years.

Sample-size-based taxon-accumulation curves (Figure 4.3) were constructed to distinguish between seasonal effects on taxon richness *per se*, and those resulting from changes in abundance. In these plots, non-overlap of the 95% confidence intervals can be used as a simple but conservative criterion of statistical difference: lack of overlap guarantees a significant difference in means at $p < 0.05$, but overlapped intervals do not guarantee a non-significant difference (Colwell *et al.*, 2012). Estimated taxon richness for autumn 2011 was significantly higher than estimates for the other three seasons across all sample sizes. In contrast, rarefaction of the autumn 2012 reference sample to a sample size comparable to those recorded in spring 2012 and 2013 yielded a significantly lower estimated taxon richness for autumn 2012. The extrapolated curves for the spring samples reached an asymptote at a much lower sample size than observed with the autumn 2012 data. As a result, differences in estimated richness between the autumn 2012 and spring samples decreased for higher sample sizes, and the extrapolated taxon richness for autumn 2012 intersected with those of both spring 2012 and 2013 at sample sizes exceeding 10,000 individuals (not shown in Figure 4.3).

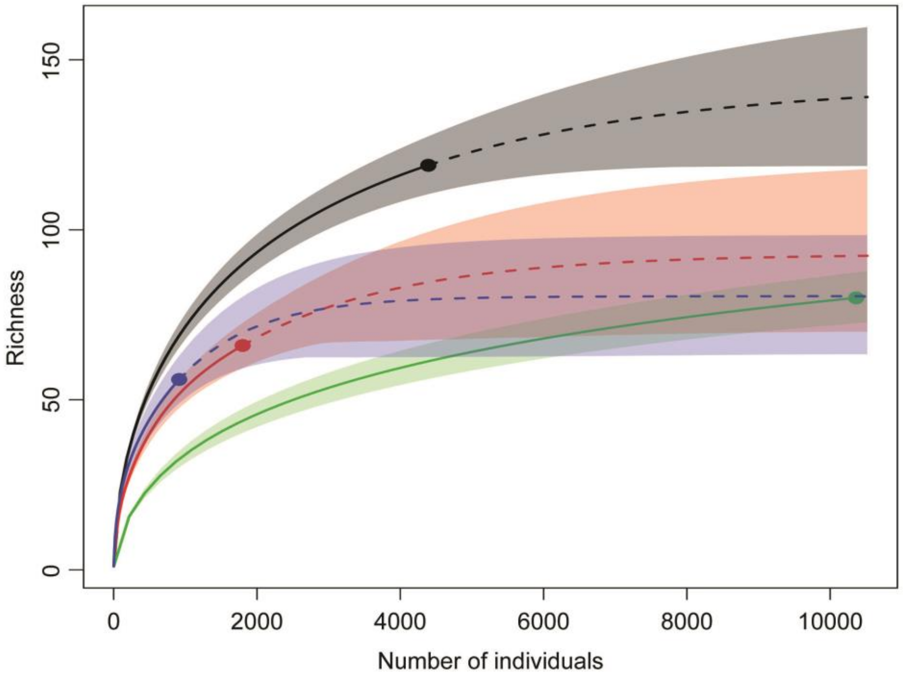

Figure 4.3 Sample-size-based taxon-accumulation curve produced via individual-based rarefaction (solid lines) and extrapolation (dashed lines) from reference samples (filled circles) for each of four seasons: autumn 2011 (black), spring 2012 (red), autumn 2012 (green) and spring 2013 (blue), together with their 95% unconditional confidence intervals (shaded areas).

The cumulative ranked species-abundance curves for the four seasons (Figure 4.4) did not indicate a seasonal trend in dominance structure, with the main difference being the greater dominance of the top five ranked species in autumn 2012 when compared to the dominance curves for the other three sampling sessions. On the other hand, multivariate analysis of the taxon-abundance matrix yielded similar results to those obtained with univariate measures of observed taxon richness and abundance, with the PERMANOVA results showing a significant seasonal effect on the assemblage structure (Table 4.2). Pair-wise tests carried out for the interaction term ‘Season x Site’ for pairs of levels of the factor ‘Season’ detected differences in assemblage composition and taxon abundance between the autumn and spring seasons at all five sites. As in the case of the univariate analyses, no significant interaction for the term

'Year x Season' was recorded. These results were corroborated by the nMDS ordination plot (Figure 4.5) where the seasonal effect was clearly evident.

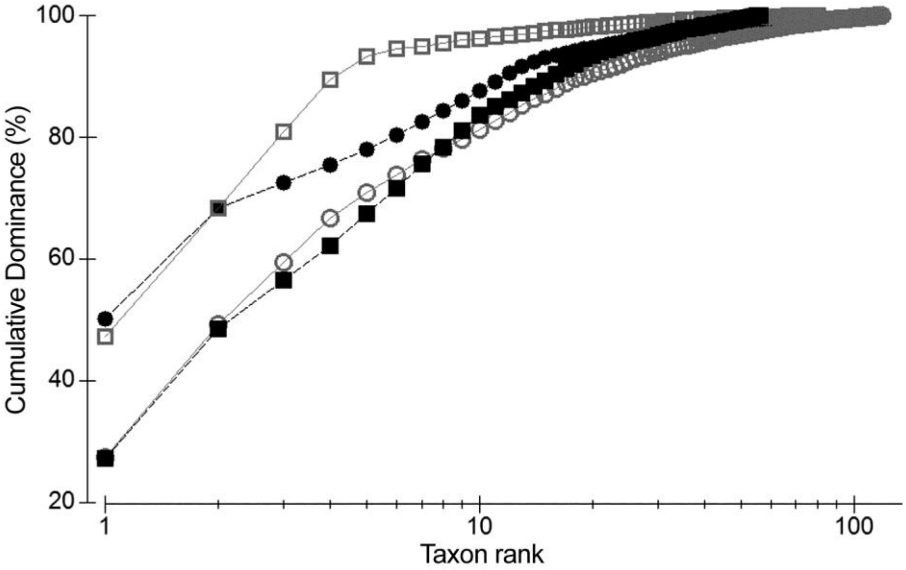

Figure 4.4 Cumulative ranked taxon-abundance curves based on season-pooled data collected in autumn 2011 (empty circles), spring 2012 (filled circles), autumn 2012 (empty squares) and spring 2013 (filled squares).

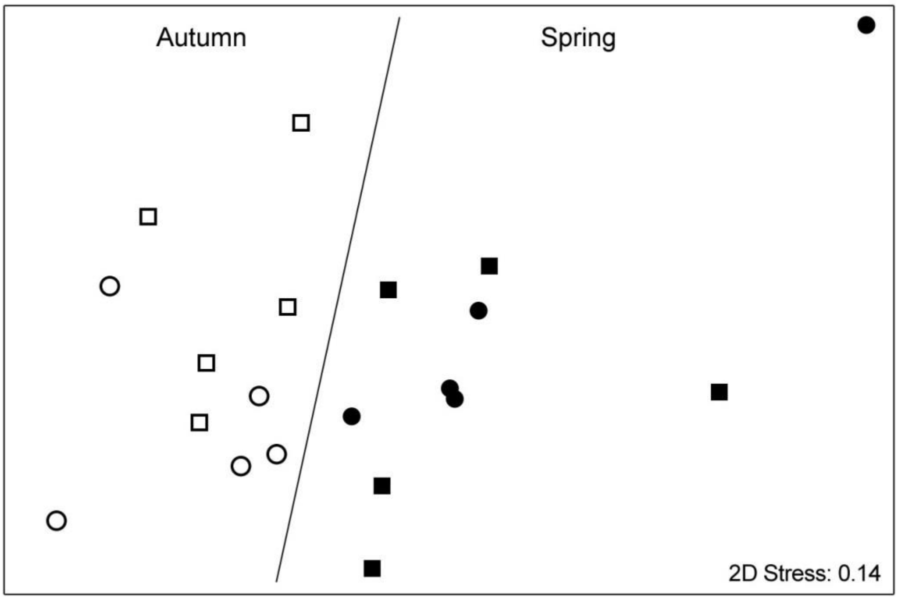

Figure 4.5 Non-metric multi-dimensional scaling ordination plot of the five sites sampled in autumn 2011 (empty circles), spring 2012 (filled circles), autumn 2012 (empty squares) and spring 2013 (filled squares), based on Bray-Curtis resemblances produced using square-root transformed abundance data.

Table 4.2 Permutational multivariate analyses of variance based on the Bray-Curtis similarity measure for square-root transformed abundance data.

(a) Global test

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P (perm)**	**Unique perms**
Year	1	9713	9713	1.4407	0.248	915
Season	**1**	**28213**	**28213**	**4.4489**	**0.016**	**928**
Site	4	42060	10515	8.6434	**0.001**	998
Year x Season	1	7109	7109	1.5200	0.226	987
Year x Site	4	26968	6742	5.5419	**0.001**	999
Season x Site	4	25366	6341	5.2128	**0.001**	997
Year x Season x Site	4	18709	4677	3.8448	**0.001**	997
Residual	60	72993	1216.5			
Total	79	2.31×10^5				

(b) Pair-wise comparisons of seasons within each site

Site	**t**	**P (perm)**	**Unique perms**
1. Xoqqa	4.3978	**0.001**	999
2. Marsaxlokk	1.9599	**0.002**	999
3. Hofra z-Zghira	3.6642	**0.001**	999
4. Qawra	2.3967	**0.001**	999
5. Tunnara	2.3919	**0.001**	998

The results of SIMPER analysis are summarised in Table 4.3; only taxa contributing more than 1% to the similarity or dissimilarity are shown. Average similarity of sites within a season ranged from 30% for autumn to 35% in spring. Taxa that are characteristic of shallow-water pebble beds, such as *Melita hergensis*, *Gammarella fucicola*, *Parhyale aquilina* and *Gibbula* spp. were the main contributors to site similarity in both autumn and spring. The dissimilarity between the two seasons was relatively high (77.2%). This was mainly driven by seasonal differences in the abundance of the aforementioned taxa. In most cases, abundances recorded in spring were lower than those recorded in autumn although some polychaete taxa (e.g. Protodrilidae sp.) showed the opposite trend.

Table 4.3 Output from SIMPER analysis, identifying main taxa contributing to: (a) the within cluster similarity for each of the two seasons, and to (b) the seasonal dissimilarities; only taxa contributing more than 1% to the similarity or dissimilarity are included.

(a) Similarities

	Taxa	**Av.Abund**	**Av.Sim**	**Contrib%**
Autumn *Average similarity: 30.35*	*Melita hergensis*	78.68	8.69	28.64
	Gibbula varia	30.25	8.29	27.31
	Gammarella fucicola	152.13	3.94	12.97
	Gibbula divaricata/rarilineata	11.65	2.03	6.70
	Leptochelia savignyi	12.00	1.82	6.00
	Clibanarius erythropus	7.53	1.54	5.06
	Parhyale aquilina	35.60	1.04	3.42
	Xantho pilipes	2.25	0.45	1.50
Spring *Average similarity: 35.86*	*Melita hergensis*	28.78	22.41	62.49
	Protodrilidae sp.	9.50	4.68	13.04
	Parhyale aquilina	5.80	1.32	3.67
	Gibbula varia	2.45	1.05	2.91
	Dorvilleidae sp. B	1.80	0.76	2.12
	Gammarella fucicola	1.00	0.70	1.95
	Jassa ocia	1.38	0.70	1.94
	Spionidae sp. A	1.70	0.61	1.71
	Gibbula divaricata/rarilineata	2.00	0.37	1.03

Table 4.3 (continued)

(b) Dissimilarities

	Taxa	**Av.Abund Autumn**	**Av.Abund Spring**	**Av.Diss**	**Contrib%**
Autumn vs Spring *Average disimilarity: 77.42*	*Gammarella fucicola*	152.13	1.00	19.02	24.56
	Melita hergensis	78.68	28.78	12.67	16.36
	Gibbula varia	30.25	2.45	9.60	12.40
	Parhyale aquilina	35.60	5.80	5.06	6.53
	Leptochelia savignyi	12.00	0.45	4.82	6.23
	Gibbula divaricata/rarilineata	11.65	2.00	4.24	5.48
	Protodrilidae sp.	1.15	9.50	3.20	4.13
	Clibanarius erythropus	7.53	2.50	3.13	4.04
	Cerithium spp.	3.08	0.08	1.41	1.82
	Spionidae sp. A	2.05	1.70	1.10	1.42

4.4 Discussion

The Mediterranean biocoenosis of infralittoral pebbles is generally considered to be impoverished (e.g. Bellan-Santini, 1985), but this may be due to inadequate biotic characterisation of these poorly-studied habitats (see Chapter 3). The present findings of over 17,000 individuals belonging to 163 macrofaunal taxa support the latter view. However, these data are based on samples collected biannually across a two-year period and seasonal differences were also evident, with appreciable reductions in the total number of taxa and total abundance occurring between autumn and spring in both years. The lowest number of taxa (56) and individuals (915) were recorded in spring 2013 (from a 2 m^2 sampling area); these values indicate that infralittoral pebble beds studied are still not depauperate in spring but are certainly more impoverished than they are in autumn.

The magnitude of seasonal differences in observed richness and abundance varied among sites, but all shared a consistent pattern of lower values for both parameters being recorded in spring. According to Grime's (1977) definition, such a reduction in abundance (and hence loss of biomass) constitutes a disturbance. On the other hand, seasonal changes in diversity and evenness measures were not consistent among sites within a particular year, or within a single site across the two years. Given that statistically significant seasonal changes were recorded in the number of taxa and total abundance, but not in those parameters that incorporate relative abundances (diversity and evenness), it may appear that proportional abundances of taxa did not vary temporally. In actual fact, the k-dominance plots indicated that some differences in taxon relative abundances were present in different sampling occasions, but these were not directly related to seasonality.

The lower number of taxa per sampled area recorded in spring samples may simply be due to the smaller sample sizes (Gotelli and Colwell, 2001). For instance, in the case of stream invertebrates, McCabe and Gotelli (2000) showed that disturbance reduced both the total abundance and number of sampled taxa, but actually led to a higher estimated richness for a given sample size. The sample-size-based taxon-accumulation curves obtained in the present study proved inconclusive in this regard: while autumn 2011 had the highest estimated richness across all sample sizes, estimated richness for autumn 2012 was lower than spring ones at low sample sizes. Furthermore, the autumn 2012 taxon-accumulation curve intersected with those for the spring sampling sessions at large sample sizes. This outcome is likely due to the differences in the dominance of the most abundant taxa, since the five most abundant taxa accounted for over 90% of the individuals present in the autumn 2012 reference sample, leading to lower richness estimates upon rarefaction. Intersection in species-accumulation curves is a common occurrence when comparing assemblages under different disturbance regimes (Lande *et al.*, 2000).

It is interesting to note that effects of disturbance varied among taxonomic groups: the PERMANOVA *p*-values for seasonal changes in observed richness and abundance were close to or below the level of significance (0.05) in the case of the Mollusca and Crustacea, but highly non-significant for the Polychaeta. This may seem counter-intuitive since polychaetes are most susceptible to being damaged, given that molluscs possess protective shells, while crustaceans are comparatively agile. However, polychaetes probably occur deeper within the pebble bed, residing as infauna in the basal sandy layers which, being buried beneath the superficial pebble layers, experience milder physical forces. On the other hand, molluscs and crustaceans occurring in the interstitial spaces between pebbles are more subject to physical disturbance. This was also corroborated by the results of multivariate analyses. Although these indicated seasonal variation in the assemblage structure of motile fauna associated with the shallow-water pebble beds, inspection of SIMPER results revealed that similarities within each of the two seasons and differences among them were driven by the same taxa, with the main difference between the two seasons being a reduction in the abundance of amphipods such as *Melita hergensis*, *Gammarella fucicola* and *Parhyale aquilina* and of trochid gastropods, notably *Gibbula* spp.

While observational studies such as this one enable description of patterns, one can only speculate with regards to the underlying processes causing these changes; manipulative experiments that control for confounding effects are necessary to enable causative inferences. Accordingly, the agents of seasonal disturbance acting on the infralittoral pebble-bed assemblages studied here cannot be determined with certainty.

Sousa (2001) provides a comprehensive list of different kinds of natural disturbance in marine benthic assemblages, including 20 different agents of physical disturbance and five kinds of biological disturbance. Of these, the physical factors “storm waves and currents”, “water or current borne sediment”, “water or current borne cobbles”, “low water temperature” and “freshwater flooding” (Sousa, 2001) are most relevant to seasonal disturbance in pebble beds, although only the first one has been implicated in previous studies on subtidal cobble habitats (Osman, 1977; Lieberman *et al.*, 1979; Davis and Wilce, 1987a). While the relative importance of individual factors cannot be ascertained, all reach peak intensities during storm events; physical disturbance during winter storms is therefore deemed to be the most likely cause of the seasonal variation observed in the present study.

In their work on intertidal boulder fields, Dean and Connell (1987b) noted that species richness, abundance and diversity of motile invertebrates were lower in small boulders that were frequently overturned. This was attributed to an indirect disturbance effect: the frequently disturbed small boulders were characterised by early successional macroalgae, which have a lower biomass and surface area when compared to the macroalgae of middle and late successional stages found on more stable larger boulders. The motile fauna differed as a result of the presence of algae having different structures (Dean and Connell, 1987b). These authors do not explain the processes leading to the large seasonal variation in species richness and abundance they recorded among small boulders, so it is not clear whether these were also due to indirect effects, or if the higher frequencies/intensities of disturbance conditions in winter directly caused loss of biomass of motile fauna.

Indirect effects as a result of changes to the sessile assemblage can be excluded in the present case, since the Maltese shallow-water infralittoral pebble beds lacked a macroalgal cover throughout the two-year study period, presumably because these pebbles are more unstable and frequently overturned. Physical agents of disturbance probably had a more direct role in determining the observed seasonal changes in the motile macrofaunal assemblage of these habitats. The main seasonal effect appears to be the decrease in macrofaunal abundance and concomitant decrease in the number of taxa per sampled area, especially in the case of crustaceans and molluscs. This could have been due to mortality (Ebeling *et al*., 1985; Dayton *et al*., 1989), or to migration to deeper more sheltered habitats (Pérès and Picard, 1964), or a combination thereof. Since abundances recorded in spring were low in spite of the fact that sampling was carried out on calm days, it appears that no rapid recolonisation of shallow-water pebble beds occurred once calm conditions resumed. However, repopulation of these habitats via settlement of new recruits or migration from adjacent habitats did occur during the calm summer months, as evidenced by the increase in richness and abundance detected in autumn compared to those recorded during the preceding spring.

Chapter 5:

Spatial patterns of species diversity within infralittoral pebble beds in the Maltese Islands

Chapter 5

Spatial patterns of species diversity within infralittoral pebble beds in the Maltese Islands

Abstract

Contrary to the prevailing view that Mediterranean shallow-water infralittoral pebble beds are impoverished, recent studies have suggested these habitats can support a rich and diverse biotic assemblage. Spatial patterns in macrofaunal diversity of pebble beds located in a 1 km^2 area within Marsamxett Harbour, Malta, were therefore studied to determine how a high regional diversity is maintained in these habitats. A mean alpha (within-site) richness of nearly 100 taxa was recorded, while a relative turnover rate of 10-16% occurred between site pairs. Multiplicative beta (between-site) diversity was around 2.5, leading to an overall (gamma) richness of over 250 taxa. Thus, both alpha and beta components contributed to a high gamma diversity. Variance partitioning indicated nearly equal contributions of spatial and environmental variables (namely depth, mean pebble size, % sand and silt, and % cover of coralline algae) to among-site compositional differentiation. No differences in turnover rates or outcomes of variance partitioning were observed when comparing taxa with different dispersal abilities. These findings indicate that alpha diversity is probably related to the small-scale structural heterogeneity of pebble beds, while the observed beta diversity is likely due to changes in environmental characteristics. The implications of these results for the conservation of pebble-bed assemblages are briefly discussed.

5.1 Introduction

Understanding the processes that determine patterns in species diversity is a long-standing goal of ecology, yet the spatial patterns of marine benthic diversity remain poorly known in several habitats (Costello *et al*., 2010). This is particularly true for deep-sea areas (Costello *et al*., 2010; Howell *et al*., 2010) but also applies to some of the more easily accessible shallow-water habitats (Coll *et al*., 2010). For instance, very few studies have been published on the ecological dynamics of shallow sublittoral pebble habitats, and these papers have mainly focused on the effect of disturbance on sessile biota (Davis and Wilce, 1987a; Lieberman *et al*., 1979, 1984; Osman, 1977). As a result, very little is known about the biological characteristics of such habitats.

In shallow waters of the Mediterranean, accumulations of pebbles and cobbles support a distinct biotic community known as the biocoenosis of infralittoral pebbles (e.g. Pérès and Picard, 1964; UNEP-MAP-RAC/SPA, 2006a). However, information on this biocoenosis is lacking, even in interpretive manuals for the identification of the different biocoenoses (e.g. Bellan-Santini *et al*., 2002; Relini and Giaccone, 2009). According to Bellan-Santini (1985) and Bellan-Santini *et al*. (1994) the biocoenosis of infralittoral pebbles is impoverished, but these authors concede that it has not really been studied. On the other hand, studies undertaken in the Maltese Islands as part of the present work (Chapter 3) indicated that pebble beds are more diverse than previously thought. Over 200 different macrofaunal taxa were recorded from pebble beds located in Marsamxett Harbour alone, but this regional species pool was more than twice as high as the mean species richness recorded from the individual sites, indicating that a large variation in species composition was present among sites.

Whittaker (1960, 1972) proposed partitioning species diversity at the regional scale (gamma diversity) into alpha and beta components to characterize different aspects of diversity in relation to spatial scale. Alpha represents the inventory component of diversity, that is, the species composition at a given local scale (e.g. at a single site); beta represents the differentiation component of diversity, or the change in species composition between sites (e.g. Magurran, 2004). Beta diversity was originally defined as the extent of differentiation of communities along habitat gradients (Whittaker, 1972), but the concept has been applied more widely to refer to the variability in species composition among sites for a given area at a given spatial scale (Legendre *et al.*, 2005; Anderson *et al.*, 2006, 2011; Legendre and De Cáceres, 2013). Beta diversity provides a direct link between the regional (gamma) and local (alpha) diversities in the geographical region of interest (Anderson *et al.*, 2011).

Partitioning species diversity into alpha and beta components provides useful insights on the mechanisms generating and maintaining diversity, and therefore has implications for conservation (Gering *et al.*, 2003). For instance, when only minor variation in species composition is present among sites, alpha will be the main contributor towards gamma. In this case, protecting a few sites may be sufficient to preserve the regional diversity, and management efforts should focus on safeguarding the processes contributing to alpha diversity (Legendre *et al.*, 2005). Conversely, when beta diversity is high, conserving gamma diversity will entail protection of multiple sites across the region (Gering *et al.*, 2003), while management efforts must ensure that the causes of spatial differentiation in species composition are preserved (Legendre *et al.*, 2005).

The processes underlying the different diversity components depend on the spatial scale at which alpha, beta and gamma are estimated. Over broad spatial scales (e.g. at the scale of the Maltese Islands) the regional species pool present depends on evolutionary and biogeographical processes (Shmida and Wilson, 1985). Within habitat patches at small scales (e.g. up to a few metres on rocky shores; Balata *et al*., 2007) biotic interactions and niche relations are important drivers of diversity (Shmida and Wilson, 1985), with local community composition depending on a complex interplay between abiotic and biotic factors (Menge and Olson, 1990). Changes in the physical characteristics of the habitat and dispersal constraints affect the variation in species composition and abundance among the local scale sites (Belyea & Lancaster, 1999; Balata *et al*., 2007) and are hence drivers of spatial differentiation.

Measures of diversity are therefore scale-dependant. Ecologists typically measure scale in terms of grain and extent (e.g. Wiens, 1989). In this context grain represents the scale at which alpha diversity is measured, whereas extent is the entire geographical area within which sampling is undertaken (Nekola and White, 1999; Barton *et al*., 2013). Alpha and gamma diversities depend, respectively, on grain and extent due to the well-known species-area curve (Connor and McCoy, 1979). The grain and extent used will also determine what beta diversity quantifies. If the geographical area of interest includes various habitats, and alpha diversity is measured at the habitat level, then beta diversity will be a measure of the between-habitat variability in species composition. On the other hand, beta will quantify the within-habitat compositional heterogeneity if the sampling extent is restricted to a single habitat (Gering *et al*., 2003).

The present study was carried out to investigate the spatial patterns of species diversity of the infralittoral pebble beds found in Marsamxett Harbour, Malta. These pebble beds lie within a geographical area of less than 2 km^2, which therefore constitutes the extent of the present study area, and hence the regional (gamma) scale used in this study. The grain of individual sites was purposely set at a scale of a few metres, such that alpha diversity would depend on local scale factors such as biotic interactions and small-scale habitat heterogeneity (Shmida and Wilson, 1985; Balata *et al*., 2007). Site intervals ranged from tens to hundreds of metres; hence the measured beta diversity represents the variation in species composition at these scales. Geographical proximity of sites leads to lack of independence in ecological variables (i.e. spatial autocorrelation), which must be taken into account when investigating relationships between diversity and environmental parameters (Legendre, 1993). In addition, high dispersal ability of the biota will tend to increase the scale of spatial autocorrelation and reduce beta diversity (Shurin *et al*., 2009). Comparing groups with different dispersal abilities can provide an insight on the extent of habitat and dispersal effects (Procheş *et al*., 2010).

Within this context, this study aims to: (i) measure the diversity of infralittoral pebble beds at the alpha, beta and gamma scales, (ii) partition the differentiation component of diversity in response to spatial and environmental explanatory variables, and (iii) compare the diversity patterns of taxon groups with different dispersal abilities, in order to make inferences on the underlying processes driving species diversity patterns within infralittoral pebble beds.

5.2 Material and Methods

Field and laboratory procedures

Previous surveys carried out along the low-lying coasts of the Maltese Islands indicated that extensive pebble beds occur as bands running parallel to certain sections of the shore in Marsamxett Harbour (see Chapter 2). These beds are characterised by a gently sloping bottom consisting of gravelly sand and silt with overlying accumulations of cobbles and pebbles within the 5–12 m depth range. Twelve sites were established within these beds using a systematic-cluster design, with some sites separated at scales of tens of metres and others at hundreds of metres (Figure 5.1). Such a design with unequal distances between sites maximises the chances of detecting spatial patterns (Fortin *et al*., 1989). The precise geographic position of the sites was determined in the field using a handheld GPS set; the Universal Transverse Mercator (UTM) coordinate system was utilised since these coordinates are expressed in metres, and can therefore be used to model spatial autocorrelation among sites.

At each site, divers measured the water depth using a digital depth gauge. Four replicate samples were subsequently collected; sample position was selected randomly, but no samples were collected towards the edges of the pebble beds to avoid edge effects (e.g. Todd and Turner, 1986). Sampling was carried out using a 0.1 m^2 circular sampler (modified from Borg *et al*., 2002) which was pushed into the substratum to delineate the sampling area and prevent escape of motile invertebrates. Cobbles and pebbles were hand-collected and transferred to a 0.5 mm mesh bag, while a small fine-mesh (1 mm mesh size) hand net was used to scoop the basal layer of finer granules. A suction sampler was simultaneously employed to reduce the risk of missing highly-motile organisms, thus ensuring quantitative samples were collected. The thickness of the pebble layer was then estimated by measuring the thickness of the exposed section in comparison to the adjacent undisturbed region, using a 30 cm ruler. Finally, a small core sample (area: 0.0016 m^2) of the granule layer and the underlying fine sediments up to a depth of 5 cm was collected for grain-size analysis. All fieldwork was carried out between July and September 2011.

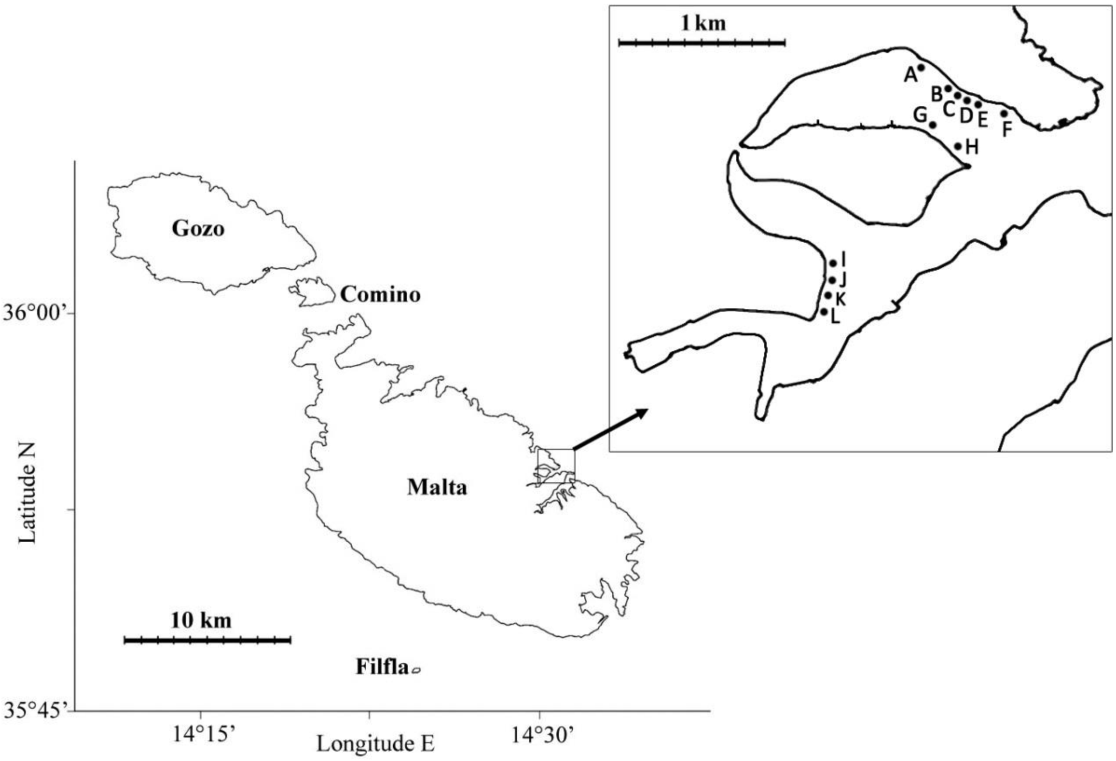

Figure 5.1 Map of the Maltese Islands with enlarged view of Marsamxett Harbour (inset) showing the location of the twelve sampling sites (A – L) used in the present study.

Samples were subsequently transported to the laboratory and preserved in 10% formaldehyde in seawater. For biotic analysis, the samples were rinsed and sorted, separating the macrofauna (retained by a 0.5 mm sieve) into major taxonomic groups, while retaining the sediment for granulometric analysis. The biota were subsequently identified to the lowest taxon possible and enumerated to construct a taxon-abundance matrix. Separate matrices for taxa with different dispersal potential were also constructed to enable assessment of differences related to dispersal abilities. Very limited information on the dispersal range of adult individuals was available; consequently, classification of taxa into “low” (58 taxa) or “high” (196 taxa) dispersal potential categories was mainly based on the presence or absence of a pelagic phase in their life cycle (Josefson and Göke, 2013).

For sediment analysis, all the pebbles greater than 8 mm were manually sorted into size classes at half-phi intervals on the Udden-Wentworth scale (i.e. eight size classes in the 8–128 mm range) using a gravel sizing template (Hydro Scientific Ltd.). The percentage cover of encrusting and filamentous algae on these larger pebbles was also recorded. Particles smaller than 8 mm were treated with sodium hexametaphosphate and wet-sieved using a 0.063 mm mesh for the determination of silt content. They were then dried and separated into size classes at phi-intervals using a series of nested test-sieves (0.063–8 mm) shaken on a mechanical sieve-shaker for 20 minutes at moderate amplitude (see Bale and Kenny, 2005). Since the finer sediment was collected using smaller corers, the masses of the particle-size fractions less than 8 mm were scaled up using the ratio of the two corers as the scaling factor.

Grain-size data were analysed via the method of moments to obtain the geometric mean particle size using the GRADISTAT program (Blott and Pye, 2001). Since bimodal distributions were common, the mean particle size for the gravel fraction (particles >2 mm) was calculated independently of any finer particles, which were then quantified separately in terms of the percentage sand and silt content. The algal cover and grain size parameters were used together with sampling depth and cobble layer thickness to construct a matrix of environmental variables.

Statistical analyses

Two indices were used to estimate inventory diversities: taxon richness and Hill numbers. The recorded number of taxa represents the observed taxon richness (S_{obs}). Estimates of the total taxon richness (S_{est}) were obtained at both alpha and gamma scales using the *Chao1* estimator (Chao, 1984), which has been shown to work well for marine benthic assemblages (Foggo *et al*., 2003). Gamma taxon richness (S_γ) was calculated based on pooled data from all sites, while alpha richness (S_α) was estimated as the mean site richness.

Hill numbers (${}^qD$) form part of a unified family of diversity indices developed by Hill (1973), which offer a number of advantages over other diversity indices (summarised by Chao *et al*., 2014). In particular, there is a direct link between Hill numbers and differentiation diversity (Jost, 2006, 2007) and there appears to be consensus on the use of Hill numbers for the purposes of diversity partitioning (Ellison, 2010). Hill numbers express diversity in terms of effective number of species ("true diversity" *sensu* Jost, 2006), and differ among themselves only in the value of the exponent q, which determines the weight given to common species. When $q = 0$ all species are weighted equally such that ${}^0D = S_{\mathrm{obs}}$, whereas higher values of q place increasingly greater weight on the most abundant species (e.g. Jost, 2006). Thus, the steepness of a diversity profile (*sensu* Tóthmérész, 1995) of ${}^qD$ versus q reflects the degree of unevenness (or dominance) in the distribution of relative abundances (Jost, 2010).

Hill numbers were therefore used to measure alpha (${}^qD_\alpha$) and gamma (${}^qD_\gamma$) diversities; these were calculated for values of q ranging from 0 to 4, enabling the construction of diversity profiles. In the case of alpha diversity, replicate data from each site were first pooled together to obtain the taxon abundance values per site. Alpha and gamma diversity were calculated using equal community weights, which is appropriate when measuring compositional differentiation (see Jost, 2007 and Chao *et al.*, 2012). Multiplicative partitioning was then used to obtain beta diversity ($^{q}D_{\beta} = {}^{q}D_{\gamma} / {}^{q}D_{\alpha}$) for the entire range of q values. Beta measured in this manner yields the effective number of distinct samples in the region, and can vary independently of alpha (Jost, 2007). Beta diversity of order zero ($^{0}D_{\beta}$) is equivalent to Whittaker's (1960) multiplicative beta (β_{w}), which can range from 1 to the number of sites (N). For comparison of differentiation diversity among taxon groups, $^{q}D_{\beta}$ was converted into measure of relative turnover rate per site ($^{q}\beta_{-1} = [{}^{q}D_{\beta} - 1] / [N - 1]$; Harrison *et al.*, 1992; Jost, 2007), which was plotted as a dissimilarity profile.

Beta diversity can also be measured from site pair-wise similarity values, through the dispersion of sites in multivariate space (Anderson *et al.*, 2006). The two methods are complementary; indeed, $^{0}D_{\beta}$ is directly related to the Jaccard and Sørenson dissimilarities (Anderson *et al.*, 2011; Chao *et al.*, 2012). The multivariate approach has two main advantages. Firstly, a test for homogeneity of multivariate dispersions (Anderson, 2006) can be used as a test of the null hypothesis of no difference in beta diversity among two or more groups (Anderson *et al.*, 2006). Secondly, the variance in assemblage composition can be partitioned as a function of environmental and spatial explanatory variables to estimate their relative contributions to the origin of beta diversity (Borcard *et al.*, 1992; Legendre and De Cáceres, 2013). Tests for homogeneity of multivariate dispersion and variance partitioning were both used to compare the beta diversity patterns of taxon groups with different dispersal abilities. Pair-wise dissimilarities were obtained using the Jaccard index, which fulfils all requirements necessary for beta diversity assessment given in Legendre and De Cáceres (2013). Multivariate dispersion was then measured as the average distance

(D_{cen}) from the twelve sampling units to the group centroid in the multivariate space. Distance-based tests were then performed via the PERMDISP routine using 9,999 permutations (Anderson, 2006).

For variance partitioning, a matrix of spatial variables was constructed using the nine terms of cubic trend surface (i.e. the UTM coordinates x and y, and the terms x^2, xy, y^2, x^3, x^2y, xy^2, y^3). This allows modelling features such as gaps or patches instead of being limited to simple linear gradients (Borcard *et al*., 1992). Distance-based multiple regression and redundancy analyses (DistLM and db-RDA; McArdle and Anderson, 2001) were used to obtain the amount of variation in biotic data ‘explained’ by the environmental or spatial matrices. In order to avoid artificial increase of the explained variation by mere chance, a model building approach using the ‘Best’ procedure was used to select those variables with the highest explanatory power according to the adjusted-R^2 selection criterion (Anderson *et al*., 2008). The selected variables were then used in partial DistLM analysis using the environmental data as explanatory variables while controlling for spatial variation and vice versa. Thus, variance in the biotic data was partitioned into four components: non-spatial environmental variation, variation due solely to spatial patterns, variation due to both environmental and spatial factors, and residual variation (see Borcard *et al*., 1992; Legendre *et al*., 2005).

Estimates of total taxon richness were obtained using the program PAST v2.17 (Hammer *et al*., 2001), while estimates of alpha, beta and gamma diversities based on Hill numbers were calculated using the equations given in Jost (2007). The PERMDISP analysis and variance partitioning were carried out using the PRIMER V6 software (Clarke and Gorley, 2006) with PERMANOVA+ v1 add-on (Anderson *et al*., 2008).

5.3 Results

A total of 19,953 individuals belonging to 254 macrofaunal taxa were recorded from the twelve sampling sites. This regional species pool consisted predominantly of molluscs (98 taxa), polychaetes (65 taxa) and crustaceans (63 taxa). The remaining taxa belonged to a variety of faunal groups, namely the Cnidaria, Platyhelminthes, Sipuncula, Nemertea, Pycnogonida, Echinodermata and Chordata. Almost one third third (31%) of the taxa were represented by only one or two individuals. Based on the ratio of these singleton and doubleton taxa, *Chao1* estimated a regional taxon richness of 301 taxa.

Mean richness values per site are given in Table 5.1. The proportion of taxa belonging to the different faunal groups did not differ significantly among sites ($\chi^2 = 23.56$; $df = 33$; $p = 0.89$). Similar relative frequencies of taxa having low or high dispersal potential were also recorded among sites ($\chi^2 = 8.62$; $df = 11$; $p = 0.66$). Gamma richness was between 2.21 and 2.75 times higher than alpha richness in nearly all cases. This equates to a relative turnover rate (${}^{0}\beta_{-1}$) of 0.11–0.16. Thus, on average there was an 11–16% change in species composition between one site and another within the region. This turnover rate was consistent among different taxonomic groups, and even between groups of taxa having a different dispersal potential.

Table 5.1 Taxon richness patterns within the infralittoral pebble-bed assemblage at Marsamxett Harbour, when considering all taxa and sub-groups classified according to taxonomical relationship or dispersal potential. Inventory richness is shown at the regional ($^0D_\gamma$) and local ($^0D_\alpha$) scales. Values are based on the recorded taxon counts, with the exception of 'Estimated Richness' which was calculated using the *Chao1* estimator. The beta component ($^0D_\beta$) was obtained by multiplicative partitioning of gamma richness and converted to a measure of relative turnover rate ($^0\beta_{-1}$).

	Inventory richness		**Richness differentiation**	
	$^0D_\gamma$	$^0D_\alpha$ **(Mean ± SD)**	$^0D_\beta = \beta_w$	$^0\beta_{-1}$
All Taxa				
Observed Richness	254	97.3 ± 11.1	2.61	0.15
Estimated Richness	301.4	136.4 ± 17.0	2.21	0.11
Taxonomic Groups				
Mollusca Richness	98	35.7 ± 6.5	2.75	0.16
Crustacea Richness	63	27.5 ± 4.5	2.29	0.12
Polychaeta Richness	65	26.3 ± 5.0	2.48	0.13
Dispersal potential				
High dispersal potential	196	75.9 ± 7.6	2.58	0.14
Low dispersal potential	58	21.3 ± 5.0	2.72	0.16

Taxon richness represents Hill diversity of order zero ($^0D_\gamma$ or $^0D_\alpha$). For a more complete picture, plots of the whole range of Hill numbers (for q values of 0–4) at both gamma and alpha scales are shown in Figure 5.2. In these plots ^1D can be interpreted as the number of "typical" taxa, whereas ^2D represents the number of "very abundant" taxa (Chao *et al.*, 2012). The regional diversity profile dropped more steeply than the alpha diversity profile. Consequently, beta diversity ($^qD_\beta$) decreased from an initial value of 2.61 at $q = 0$ to a minimum of 1.47 at around $q = 1.6$. The proportion of "typical" and "abundant" taxa changing among sites was 4–5% (Figure 5.3).

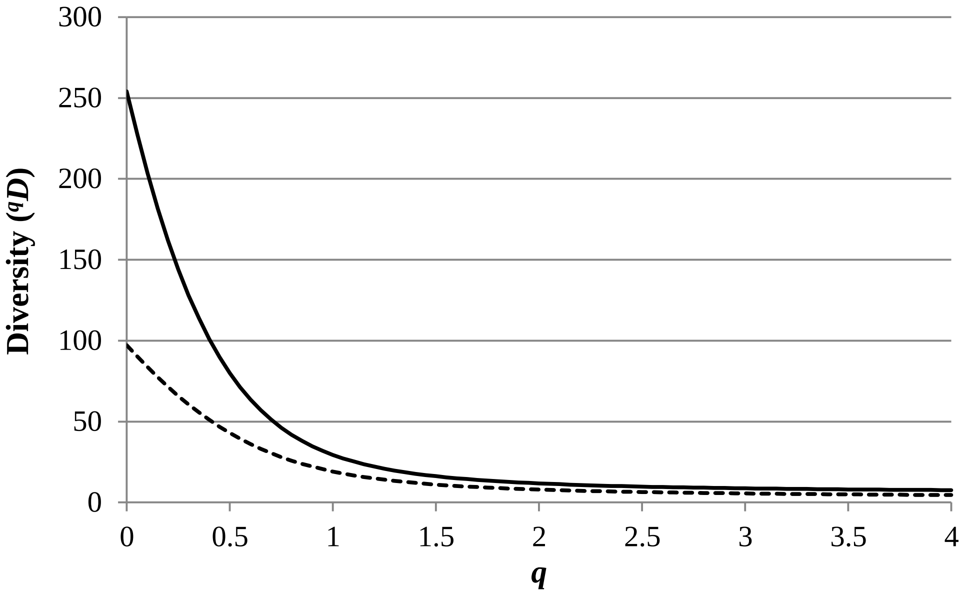

Figure 5.2 Diversity profiles of the pebble-bed assemblage in Marsamxett Harbour at the regional (solid line) and local (dashed line) scales. Each profile consists of a plot of Hill numbers (^qD) for values of q ranging from 0 to 4; as q increases, more weight is given to common species in the analysis.

No clear differences were evident in the relative turnover rate of taxa having a low or high dispersal potential (Figure 5.3). The turnover rate for taxa with a low dispersal potential was slightly higher at $q = 0$ and $q \geq 2$, but lower at $q = 1$. Both plots were also very similar to the turnover profile of all taxa taken together. Similar results were obtained via comparison of multivariate dispersion patterns. In terms of Jaccard similarities, dispersion of sites in multivariate space based on taxa having a low dispersal potential ($D_{\text{cen}} = 40.6$) was only marginally higher than that for taxa with a higher dispersal potential ($D_{\text{cen}} = 39.4$). This difference was not statistically significant ($F_{1,22} = 0.27$; $p = 0.646$).

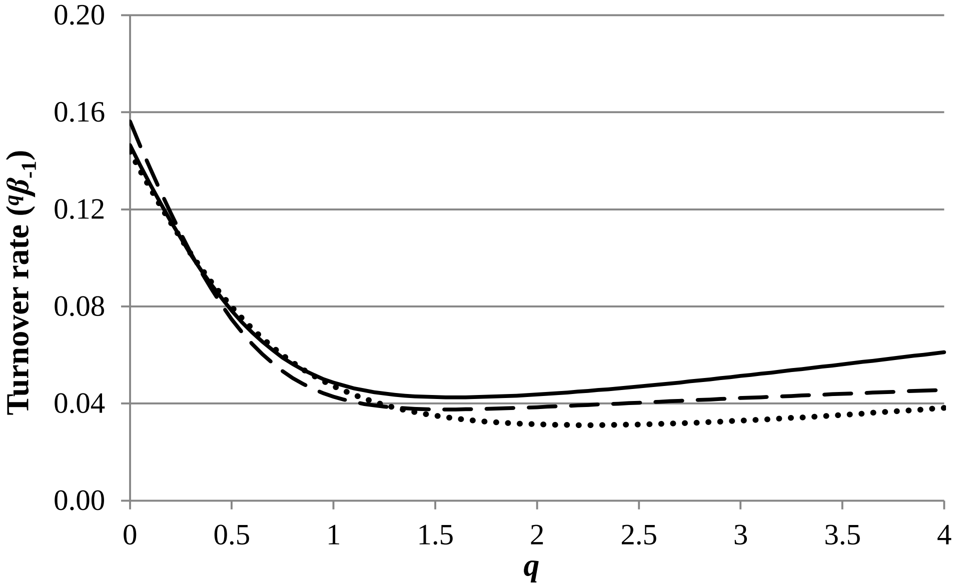

Figure 5.3 Dissimilarity profile of relative turnover rate per site ($^{q}\beta_{-1}$), obtained via multiplicative partitioning of the regional diversity; values of the exponent q (plotted on the x-axis) control the weight given to common species in the analysis. Separate plots for all taxa taken together (solid line) and sub-groups of taxa having a low (dashed line) or high (dotted line) dispersal potential are shown.

Multivariate multiple regression using environmental data as the explanatory variables for the patterns in taxon composition among sites (based on Jaccard similarities) yielded a parsimonious model including four variables: depth, mean pebble size, percentage sand and silt content, and percentage cover of crustose algae. The constrained ordination obtained by db-RDA is shown in Figure 5.4. The first two axes account for 64.5% of the fitted variation, but only 30.7% of the total variation in taxon composition. Overall, these four environmental variables ‘explained’ 45.1% of the total variation in biotic data.

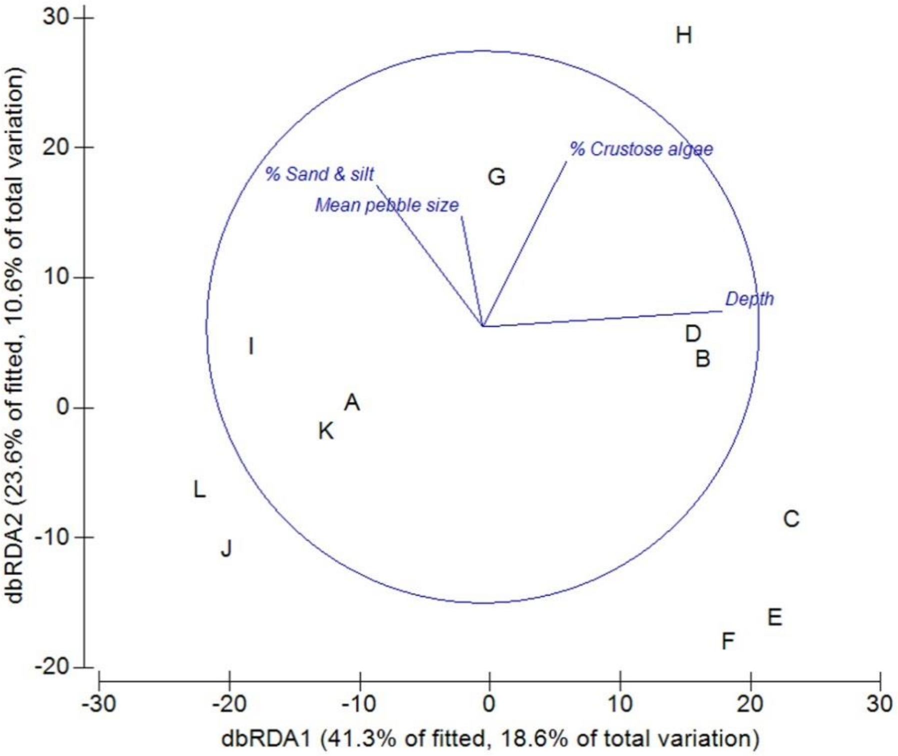

Figure 5.4 Distance-based RDA ordination relating environmental variables to the biotic data, with vector projections of the physical factors selected by the DistLM routine. Length and direction of the vectors represent the strength and direction of the relationship. Ordination was based on pair-wise similarities in biotic composition among the twelve sites (A – L; see Figure 5.1) measured using the Jaccard similarity index.

When using spatial data, the DistLM 'Best' procedure selected four of the nine terms included in cubic trend surface equation; these accounted for 47.6% of the variation in biotic composition among sites. When considered simultaneously (via partial analysis) the spatial and environmental parameters explained 79.2% of the total variation in biotic data. The resulting partitioning of variation in taxon composition between the two explanatory matrices is shown in Figure 5.5. Approximately 13.5% of the variation was due to both spatial and environmental variables, while 20.8% remained unexplained.

Figure 5.5 also shows the variation partitioning obtained when the analyses was based on groups of taxa having different dispersal potential. The relative contribution of spatial variables alone (31–35%), environmental variables alone (28–33%) and both space and environment together (12–17%) to the variation in composition of the 'low dispersal' and 'high dispersal' groups was very similar, with around 20–24% of the variation remaining unexplained in both cases.

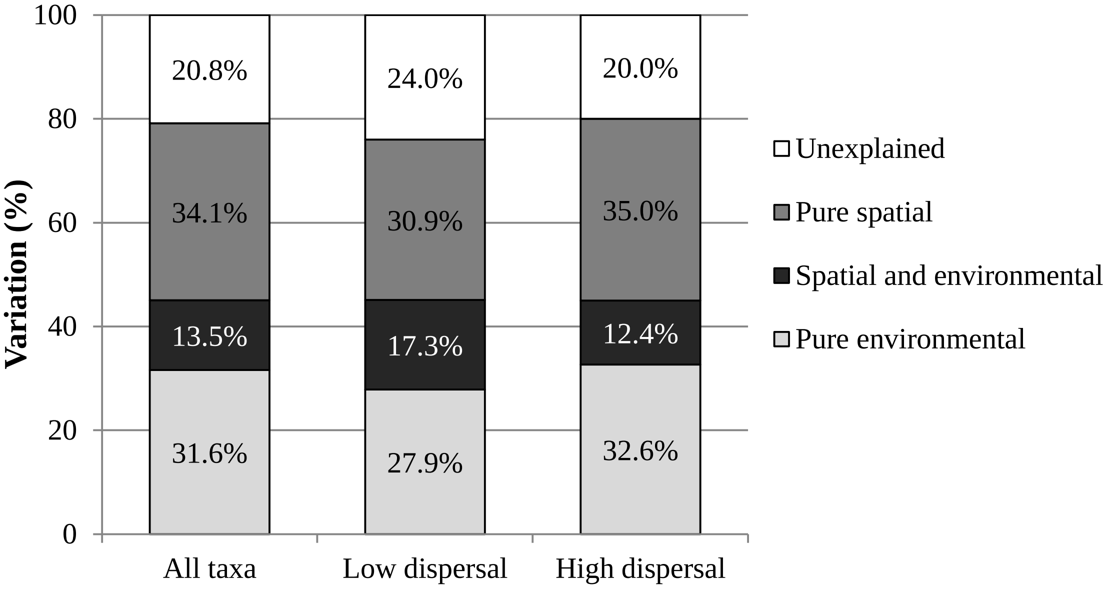

Figure 5.5 Variation partitioning of diversity in taxon composition (beta diversity) among sites, as measured using the Jaccard similarity index. Partitioning was obtained via DistLM analyses using spatial and environmental variables as predictors.

5.4 Discussion

This study recorded a high diversity of macrofaunal taxa in pebble beds found within Marsamxett Harbour, corroborating the results of previous surveys in this habitat (Evans, 2007; Chapter 3). At the scale of a single site, a mean of nearly 1,700 individuals belonging to just under 100 taxa were present in total among four 0.1 m^2 replicate samples. At such fine spatial scales, diversity depends on the interactions among species and between species and their environment (Shmida and Wilson, 1985). Factors that (i) increase the resource spectrum available for different species to exploit, (ii) reduce niche breadth via increased resource partitioning, or (iii) enable greater niche overlap among species, can all lead to a higher richness (MacArthur, 1972).

Quantifying small-scale habitat features was not the focus of the present study, but it is clear that the high alpha richness is partly due to the structurally complex nature of pebble-bed habitats (Robinson & Tully 2000a, 2000b). The pebble beds in Marsamxett consist of accumulations of pebbles overlying a middle stratum of granules and sand, while a basal silty layer is also present (Chapter 2). Thus, these pebble beds are characterised by fine-scale heterogeneity, enabling them to provide a wide range of important resources such as space and food (Shmida and Wilson, 1985).

The larger pebbles offer a hard surface for epifaunal species (e.g. spirorbid polychaetes), while the wide range of sediment particle sizes results in interstitial spaces of various dimensions. Consequently, the interstitial fauna ranged in size from a few centimetres (e.g. xanthid crabs) to a millimetre (e.g. cystiscid molluscs). In addition, the sand and silt layers are suitable substrata for burrowing infauna (e.g. bivalves). Meiofauna living in the interstitial spaces between finer particles were not considered in the present study, but these would further increase the local scale richness if included. This variety of microhabitats provides living space and refugia from predators for animals of different sizes (Linnane *et al.*, 2003).

In addition, the pebble-bed macrofauna can utilise a wide range of food sources. Filamentous and coralline algae form the basis of herbivorous food chains, but each offers a different type of food resource requiring specialised grazing mouthparts to consume, enabling resource partitioning (e.g. between chitons and trochid gastropods) (see Steneck and Watling, 1982). Given the relatively large interstitial spaces found between the larger pebbles forming the upper layers of the pebble bed, water circulation is likely to be sufficient to provide a constant supply of seston for suspension feeders. Indeed, the porcelain crab *Pisidia bluteli*, a suspension feeder (e.g. Achituv and Pedrotti, 1999), was one of the most abundant species found living interstitially within the pebble beds. Detritus trapped within the complex structure of the pebble beds also serves as an important food source for deposit feeders. Grazers, suspension feeders and deposit feeders occupy the lower trophic levels in marine food webs and serve, in turn, as the food resource for predatory organisms.

Furthermore, pebble beds are dynamic environments subject to physical disturbance, as demonstrated by the paucity of erect macroalgae on the surface of the pebbles. Hydrodynamic forces during stormy weather are probably the major cause of pebble overturn during winter months, but may play a minor role during prolonged periods of calm weather that can occur in summer. Field observations made whilst sampling indicated that bioturbation, especially by the striped red mullet *Mullus surmuletus*, may be another important cause of disturbance. Divers are also known to frequent the Marsamxett Harbour area to collect the edible warty venus *Venus verrucosa*, from beneath the pebble layer and in so doing disturb the pebble bed. Such periodic disturbance may also increase local scale taxon richness by preventing competitively dominant species from excluding weaker competitors. This effect is known to occur in the case of macroalgae and sessile epifaunal species that occur on larger cobbles (Osman, 1977; Davis & Wilce, 1987a) but its relative importance for maintaining richness of motile macrofauna in pebble beds is not known.

Spatial heterogeneity in habitat characteristics and environmental conditions across broader scales can lead to changes in species composition among sites. These habitat effects are broadly termed ‘niche-based’ processes, since they can have both direct effects by changing resource availability or indirect ones by altering interspecific interactions (Menge and Olson, 1990; Poff, 1997; Smith and Lundholm, 2010). On the other hand, ‘neutral models’ (e.g. Hubbel, 2001) show that dispersal limitation can also account for observed species distribution patterns even in the absence of environmental control. In the present case, around 28–33% of the total variation in species composition among sites was ‘explained’ by environmental variables alone. Factors such as mean pebble size, percentage sand and silt and percentage cover of crustose algae, which were selected by the multivariate multiple regression, may influence the type of resources available at a given site. Note, however, that these factors could also be acting as proxies for other unmeasured variables, so they should not be interpreted as being necessarily causative (Anderson *et al.*, 2008).

Approximately 47–48% of the variation in taxon composition within the pebble beds was attributed to spatial factors. This represents variation due to spatially structured environmental variables, or due to other ecological mechanisms resulting in spatial autocorrelation such as dispersal effects (e.g. Legendre *et al.*, 2009). The relative contribution of niche and neutral processes cannot be directly inferred from the proportion of pure spatial variation (31–35% in this case) since this may have resulted from spatially-structured environmental variables that were not included in the analysis (Anderson *et al*., 2011). However, comparing sub-groups of taxa having a different dispersal potential can provide insights into the relative importance of habitat factors and dispersal limitations (Procheş *et al*., 2010). Since dispersal is a spatial process, the spatial component of variation in species composition is expected to be higher for taxa having lower dispersal potential (Smith and Lundholm, 2010).

Profiles of relative turnover rate of taxon groups having ‘high’ or ‘low’ dispersal potential were very similar, while no significant differences in the multivariate dispersion of these two groups were recorded. In addition, no appreciable differences in the total amount of variation in assemblage composition explained by space (47–48%) or by environmental features (≈45%) were observed when comparing the two groups. The main difference was an increase in the proportion of overlapped variation, attributable to both spatial and environmental features, in the case of taxa having a low dispersal potential. This outcome implies that differences in dispersal potential were not leading to differences in spatial distribution patterns. Given that this study covered a spatial extent of under 1 km^2, the most likely interpretation is that neutral processes do not play a major role in determining the patterns in species composition within pebble-bed sites separated by tens to hundreds of metres.

Overall, the differentiation rate in taxon composition among site pairs was measured at around 11–15%. When more weight was given to common species (i.e. for $q > 0$) relative turnover rates decreased to around 4–5%. This reflects the fact that at a given spatial scale, the more abundant organisms tend to be more ubiquitous and hence exhibit lower differentiation rates; such organisms typically have wide resource use

(Barton *et al*., 2013). The relatively high rate of taxon turnover in space resulted in a comparatively high beta diversity, with regional taxon richness being around 2.5 times higher than that at a given site. This, coupled with the high alpha richness, contributes to the overall high gamma diversity recorded from the pebble beds. Gamma richness was estimated at a total of just over 300 taxa; this estimate is similar to that obtained in Chapter 3, where sampling effort was 50% of that used in the present study.

The high regional diversity of pebble beds has implications for conservation efforts. Thus far, pebble beds have been considered to be impoverished habitats (Bellan-Santini, 1985; Bellan-Santini *et al*., 1994) and hence omitted from lists of priority habitats (UNEP-MAP-RAC/SPA, 2006b), but the high species diversity recorded from pebble beds in recent studies (Chapter 3; this study) indicates that they have a greater conservation value than generally thought. The present findings on the spatial patterns of diversity have provided insights on the mechanisms that maintain this high regional diversity. Such information will serve to guide management decisions. For instance, variance partitioning indicated a high degree of spatial organisation and environmental control of beta diversity, with only 20% of the variation deemed to be due to stochastic processes and remaining ‘unexplained’. In such a situation, preserving the spatial organisation and species-environment relationships is necessary to ensure long-term maintenance of diversity (Legendre *et al*., 2005) since alteration of environmental conditions can lead to a significant reduction in beta diversity, and hence a severe loss in the overall species diversity within the region (Balata *et al*., 2007).

Chapter 6:

Discussion

Chapter 6

Discussion

6.1 Introduction

The genesis of this work was the observation of pebble beds in Marsamxett Harbour whose characteristics were not consistent with those of the biocoenosis of infralittoral pebbles as described in habitat manuals (e.g. Bellan-Santini *et al.*, 1994). In particular, these beds occurred at depths of a few meters rather than a few decimetres, the pebbles had a relatively high coralline algal cover, and the molluscan assemblage was not impoverished (Evans, 2007).

This led to a number of ecological questions regarding infralittoral pebble beds: Do different types of pebble beds exist? Where do they occur? How do they differ in terms of their physical and biological characteristics? Are pebble beds really impoverished habitats, as suggested in the literature? How does the structure of the macrofaunal assemblage of pebble beds vary in space and time? Are there correlations between patterns of variation in assemblage structure and environmental variables? What are the ecological mechanisms underlying any such patterns?

Literature on the ecology of infralittoral pebble beds is scant, and many of these questions do not appear to have been addressed in published studies. Furthermore, none of the studies available in the mainstream literature were carried out within the Mediterranean region. The present study was therefore undertaken to characterise the infralittoral pebble-bed assemblages found in the Maltese Islands in order to provide insights into the ecological dynamics of this assemblage. The main outcomes of this study are discussed below.

6.2 Characterisation of infralittoral pebble-bed assemblages

Infralittoral pebble beds are a relatively rare habitat around the Maltese Islands. Coastal surveys undertaken to map these beds identified only 15 locations with pebble beds that extended over an area of at least 25 m^2. Smaller patches with accumulations of pebbles and cobbles also occurred within these locations and elsewhere around the coast. However, these were deemed too small to be included as study sites. In addition, because of their small coverage, the total area of infralittoral pebble habitats is much lower than that covered by other infralittoral habitats such as sandy or rocky bottoms, or seagrass meadows (Borg and Schembri, 2002).

A preliminary classification of pebble-bed types based on seascape factors suggested four categories. Statistical analysis of the measured physical and biological parameters, however, indicated that two of these categories shared similar environmental features and supported similar species assemblages. They can therefore be considered to belong to the same pebble-bed type, yielding three pebble-bed types in all.

The first bed type occurred in very shallow waters (<2 m) within small rocky coves. These beds essentially represent the biocoenosis of infralittoral pebbles as described in habitat manuals and included species characteristic of this biocoenosis such as the amphipod *Melita hergensis*, the crab *Xantho pilipes* and trochid gastropods, particularly *Gibbula* spp. (Pérès and Picard, 1964; Pérès, 1967; Bellan-Santini, 1985; Bellan-Santini *et al*., 1994). Other abundant species which are not listed in biocoenotic descriptions included the amphipod *Gammarella fucicola*, the hermit crab *Clibanarius erythropus* and spirorbid polychaetes.

Such beds may be physically similar to the British biotope of highly mobile sublittoral shingle (cobbles and pebbles) described by Connor *et al*. (2004). Both habitats are thought to be impoverished as a result of physical disturbance (Bellan-Santini, 1985; Connor *et al*., 2004). However, in the present study over 17,000 individuals belonging to 163 different taxa were recorded from shallow pebble beds (total sampling area: 8 m^2) showing that the studied beds were far from being impoverished. Seasonal disturbance caused a significant reduction in taxon richness and total abundance, with lower values of both parameters being recorded in spring sampling sessions. Repopulation of these habitats via settlement of new recruits or migration from adjacent habitats occurred during the calm summer months, as evidenced by the increase in richness and abundance detected in autumn compared to those recorded during the preceding spring.

The other two pebble-bed types recorded from the Maltese Islands occurred in deeper waters, generally within the 5–12 m range. They differed from shallow beds in having a smaller mean pebble size, a lower content of sand and silt and a greater extent of algal cover on the surface of the pebbles. They were also more diverse, with mean taxon richness and diversity values being around twice as high as those of shallow beds. The percentage abundance of faunal groups recorded from the two deeper bed types was similar to that reported by Linnane *et al*. (2001) from cobble beds found at similar depths (7–17 m) in Norway, UK and Italy.

One of these pebble-bed types occurred within creeks or in the inter-matte region of *Posidonia oceanica* beds. This is not the first time that accumulations of pebbles have been noted in such environments (e.g. Colantoni *et al*., 1982; Linnane *et al*., 2001), indicating that such pebble bed-types may be widespread in the Mediterranean. When compared to other bed types, the biotic assemblage present within the seagrass/creek beds was characterised by a higher abundance of the amphipods *Maera grossimana* and *Microdeutopus* spp. and of serpulid polychaetes. However, the assemblage composition was in fact somewhat intermediate between pebble beds found in shallower sites and those found in harbour environments (see below), with many of the more common species being present in one or both of these other pebble-bed types.

The third pebble bed-type recorded from the Maltese Islands occurred in Marsamxett Harbour. These beds were characterised by a higher content of fine particles belonging to the sand and silt fraction, most likely due to lower levels of entrainment of such fine particles given the more sheltered harbour conditions (Parker and Klingeman, 1982). The pebbles also had a higher percentage cover of encrusting coralline algae. The harbour assemblage differed from the others due to the higher abundance of the porcelain crab *Pisidia bluteli*, of the gastropods *Alvania* spp. and *Bittium reticulatum,* and of the ophiuroid *Amphipholis squamata*. On the other hand, comparatively low numbers of Spirorbinae were present, possibly due to the competition for space with encrusting algae. *Pisidia bluteli* is listed by Pérès and Picard (1964) and Pérès (1967) as occurring in crevices within infralittoral hard substrata and invading pebble beds when these consist of larger pebbles. Harbour sites, however, had slightly smaller pebbles than elsewhere, although these are likely to be subjected to less physical disturbance given their sheltered location.

Neither ‘seagrass/creek’ nor ‘harbour’ bed types fit neatly within existing habitat classification schemes for the Mediterranean region (UNEP-MAP-RAC/SPA, 2006a), which may therefore require revision. The three pebble-bed types recorded in the present study can be classified under Category III.4 “Stones and pebbles” which should therefore be retained. However, in order to reflect the existence of three different pebble assemblages, sub-category III.4.1 “Biocoenosis of infralittoral pebbles” would need to be replaced by a “Biocoenosis of infralittoral pebbles in exposed shallow waters”, while another two sub-categories should be added to account for the seagrass/creek and harbour pebble-bed assemblages described above. This would bring the classification scheme for infralittoral pebbles in line with those for other mobile substrata, each of which contains more than one biocoenosis. Any changes to the UNEP-MAP-RAC/SPA scheme as suggested above could then be reflected in the EUNIS scheme.

The role of disturbance in determining assemblage structure of the two deeper pebble-bed types was not investigated in the present work. In microtidal areas such as the Maltese Islands, the hydrodynamic regime at a given site ultimately depends on its exposure to wave action and on the depth (due to wave energy depth attenuation). This implies that the magnitude of disturbance is likely to be lower in deeper beds, and even more so in sheltered harbour sites. Since higher energy environments lead to higher rates of fine particle entrainment (Parker and Klingeman, 1982) the sand and silt content can serve as a useful proxy for hydrodynamic regime. In fact, shallow sites had the lowest content of sand and silt and largest mean pebble size, while pebble beds located in harbours had the highest content of fine sediment. Thus, it is expected that in deeper sites, the effects of seasonal disturbance will be of a lower magnitude than those recorded in shallow pebble beds. The role of bioturbation in mediating disturbance remains to be evaluated.

The percentage content of sand and silt, and by inference the hydrodynamic regime, was one of the main physical factors accounting for differences in the species assemblage composition among the three pebble-bed types. Other variables correlated with biotic patterns across sites included the percentage cover of coralline algae, patch area, depth, pebble layer thickness and water temperature. Most of these parameters were also shown to be associated with the variation in assemblage structure among sites within Marsamxett Harbour, suggesting that changes in such environmental characteristics are important determinants of pebble-bed assemblage structure over a broad range of spatial scales.

6.3 Other noteworthy outcomes of this study

The main aim of this work was to characterise the pebble-bed assemblages as detailed above. Accordingly, the individual chapters included in the main body of the thesis present studies that focus on the entire assemblage. Two publications arising out of this work, however, concern individual species recorded from these pebble beds (see publications at end of thesis). The outcome of this work is summarised below.

New records of the Maltese top-shell

The Maltese top-shell, *Gibbula nivosa*, is a critically endangered marine trochid gastropod (Schembri *et al*. 2007; Evans *et al*. 2010) endemic to the Maltese Islands (Ghisotti 1976; Giannuzzi-Savelli *et al*. 1997). Although this species has been reported many times from Malta, it is rare and no live individuals were recorded between 1981 and 2006, despite intensive searches for the species in localities where it used to occur (Schembri *et al*. 2007). In 2006, a population of *G. nivosa* was discovered within

Sliema Creek, Marsamxett Harbour (sites 8–11 in Figure 2.1), while two individuals were also recorded off south-west Comino (Evans *et al*. 2010). Although the main habitat of the Maltese top-shell has been considered to be leaves of the seagrass *Posidonia oceanica*, it has also been reported from under stones in shallow water (Cachia *et al*. 1991), and the live individuals discovered by Evans *et al*. (2010) were all recorded from such infralittoral pebbles, indicating that pebble beds may be a more important habitat for *G. nivosa* than previously thought.

Given its restricted geographical distribution and rarity, *G. nivosa* is protected under both local and international legislation. Proper conservation and management of this species is necessary to safeguard it. However, very limited information on its current status exists, a situation that can only be addressed by intensive sampling of the coastline where reported habitats of *G. nivosa* occur. The present study on infralittoral pebble beds around the Maltese Islands provided an opportunity to assess the current status of this species. Live *G. nivosa* were recorded from three new locations: Hondoq ir-Rummien, Xatt l-Ahmar and Fomm ir-Rih (see Table 2.1 and Figure 2.1), with mean (±SD) densities of 2.0 ± 2.7, 0.5 ± 0.6 and 11.0 ± 13.4 ind./0.1 m^2, respectively. Given the restricted size of the pebble patches at the three sites, none of which exceeded an area of 50 m^2, estimated population sizes were low, ranging between 250 and 4400 individuals.

Gibbula nivosa is now known to occur in five separate locations around the Maltese Islands, in all cases within accumulations of cobbles and pebbles, which therefore seem to be the main habitat for this species. Past records from seagrass meadows may have resulted from collection of individuals during foraging excursions on the seagrass blades. This implies that past searches may have been unsuccessful because they were not made in the gastropod's primary habitat. Nevertheless, the present results also indicate that the Maltese top-shell is still a very rare species, being recorded from only three new sites which, together with its very narrow geographical range (the Maltese Islands), renders it highly vulnerable to extinction. Given that pebble beds appear to be its main habitat, it is pertinent to point out that *Gibbula nivosa* is listed in Annexes II and IV of the European Union's 'Habitats Directive'. Annex II includes "animal and plant species of community interest whose conservation requires the designation of special areas of conservation". Thus, the presence of this species alone provides justification for calls to include pebble beds within Maltese MPAs.

Three new records of Gobiidae from Malta

Gobies are small teleost fish that include some of the smallest marine fish species, including the smallest known fish in the Mediterranean (*Speleogobius trigloides*) (Kovačić and Patzner, 2011). The family Gobiidae has the highest species richness among fish families in the Mediterranean, comprising more than 60 species (Quignard and Tomasini, 2000; Kovačić and Patzner, 2011). However, the patterns of actual species distribution of Mediterranean gobies are still unknown for most species (Kovačić and Patzner, 2011). The situation is compounded by the fact that identification of many Mediterranean gobiid species is difficult due to their small adult size. Additionally, many gobies are cryptobenthic species that are difficult to sample. Thus many of them are still poorly known and, until recently, considered to be extremely rare (Patzner, 1999; Kovačić and Patzner, 2011).

The methods used in the present study on infralittoral pebble-bed fauna enabled collection of very small juvenile gobies, some of them just 10 mm long, from cryptobenthic habitats. These included *Gobius couchi* (single specimen collected from Tigné), *Millerigobius macrocephalus* (three individuals collected from Manoel Island and one from Tigné) and *Zebrus zebrus* (one individual collected from Mistra and another from Ta' Xbiex). In the case of *M. macrocephalus* and *Z. zebrus*, the collected specimens included the smallest known juveniles of these species to date. These records enabled examination of juvenile morphology of the three species to provide diagnostic features for identification at such small specimen sizes.

The new records from Malta represent a considerable southwards extension of the known geographic range of *G. couchi* and *M. macrocephalus*, while for *Z. zebrus* the new record connects the previously known distributions of this species in western and eastern Mediterranean. These results suggest that the gobiid diversity of Malta could be rich and similar in composition to the northern Mediterranean areas described by Kovačić and Patzner (2011). In addition, the southwards extension of the known range of two of the species indicates that their distribution is more widespread than previously thought, suggesting that they might also be recorded from the middle-south part of the Mediterranean if proper sampling for such cryptobenthic species is undertaken there.

6.4 Implications of the present work

The general introduction of this thesis provided the rationale for characterisation of benthic assemblages, highlighting the role of such studies in providing information that can aid understanding of the ecology of the system as well as guide management decisions. The ecological inferences that can be drawn from the present work and its implications for conservation are discussed below.

Ecological inferences

A total of 62,742 individuals belonging to 360 different macrofaunal taxa (Appendix A) were recorded during the course of the present study, from a total sampling area of 16 m^2. The ecological mechanisms enabling such a high diversity can be divided into two: those that contribute to a high richness in a given site (alpha diversity), and those responsible for variation in species composition among sites (beta diversity) (Whittaker, 1960, 1972). In autumn 2011, the mean richness per site in the studied pebble-bed assemblages ranged from around 45 in shallow beds to 89–99 taxa in deeper sites, whereas the total number of taxa present in any one of the three assemblages was 2–3 times higher than the mean site richness. Thus, both alpha and beta components contributed to the overall diversity of infralittoral pebble-bed assemblages.

Mechanisms operating at the scale of a single site are broadly termed niche relations (*sensu* Schmida and Wilson, 1985) because they include processes that increase the resource spectrum, reduce niche breadth or allow niche overlap (MacArthur, 1972). Biotic interactions such as competition which could reduce niche breadth are difficult to measure directly (Borcard and Legendre, 1994) and were not quantified in the present work. On the other hand, characterisation of the physical structure of pebble beds showed that a high degree of small-scale spatial heterogeneity is present, which is likely responsible for increasing the resource spectrum available for species to exploit. For instance, the architectural structure and complexity of the pebble beds provides a wide range of microhabitats than enable coexistence of various epifaunal, interstitial and infaunal species (Robinson and Tully, 2000a, 2000b; Linnane *et al*., 2001). A wide range of food resources is also available, including filamentous and coralline algae for different grazers, seston for suspension feeders and detritus for deposit feeders.

The disturbance regime may allow increased niche overlap by preventing competitively dominant species from excluding weaker competitors, although if disturbance is too severe it could lead to a reduction in diversity (Osman, 1977; Connell, 1978). The absence of erect macroalgae in the studied pebble beds indicates that such disturbance may indeed play a role in determining diversity of these assemblages. The frequency and magnitude of disturbance is higher in shallow pebble beds, potentially leading to the lower alpha richness recorded from these sites. In addition, disturbance varies seasonally, particularly in these shallow sites, and thus plays a role in mediating temporal changes in the assemblage structure of motile macrofauna present within these pebble beds. Such effects have previously been documented only for sessile biota (Osman, 1977; Lieberman *et al.*, 1979). The extent of seasonal variation in assemblage structure within deeper pebble beds remains a subject for future investigation.

Variation in environmental characteristics among sites may not only affect their alpha richness but could also result in differences in their species composition and thus contribute to beta diversity. In addition to such niche-based processes subject to environmental control (e.g. Legendre *et al.*, 2005), beta diversity could also arise via neutral processes based on dispersal limitation (e.g. Hubbel, 2001). Spatially structured environmental variables and dispersal effects both lead to spatial autocorrelation among sites (Nekola and White, 1999; Legendre *et al.*, 2009), but not all environmental variables are spatially structured.

When comparing the distribution of the three pebble-bed types recorded around the Maltese Islands, it is clear that spatial autocorrelation is generally low for the shallow and seagrass/creek assemblages. For instance, sites Wied ix-Xoqqa A and Wied ix-Xoqqa B were located within the same creek only a few tens of metres apart yet were included in separate clusters, each with more distant sites (e.g. Wied ix-Xoqqa A with Tunnara; Wied ix-Xoqqa B with Mistra). This suggests that environmental control may be a more important factor than dispersal limitation. Such an inference is corroborated by the fact that clustering of sites based on the measured physical variables yielded very similar site groups to those obtained based on biotic composition.

In the case of the pebble-bed assemblage found in harbour environments, all sites were located within Marsamxett Harbour. Thus, clustering of such sites together may have been due to their geographical proximity, whether as a consequence of niche or neutral processes. However, it was not possible to assess the relative role of environmental versus dispersal effects in giving rise to a distinct harbour pebble-bed assemblage, since this can only be done if pebble beds in harbours other than Marsamxett are also characterised. Nonetheless, this Marsamxett Harbour assemblage was associated with physical parameters such as the high fine particle content, area of the pebble bed and cover by coralline algae.

On the other hand, it was possible to analyse the contribution of environmental and spatial factors to diversity patterns within Marsamxett Harbour. Variance partitioning indicated that 45% of the variation in assemblage composition could be accounted for by environmental variables such as mean pebble size, percentage sand and silt and percentage cover of corraline algae. Spatial variables alone accounted for a further 34% of the beta diversity patterns, which could have been due to dispersal effects or to spatially-structured environmental variables that were not included in the analysis (Anderson *et al*., 2011). However, comparison of the partitioning obtained when considering taxa with different dispersal potential indicated that neutral processes did not play a major role in determining the patterns in species composition within pebble-bed sites in Marsamxett Harbour.

The observational approach used in the present work has helped in identifying spatio-temporal patterns of variation in physical and biological characteristics of infralittoral pebble beds around the Maltese Islands. Recognition of such patterns provides the basis for a better understanding of the underlying mechanisms, enabling the formulation of hypotheses about the causes of these patterns (Underwood *et al*., 2000). In the case of the studied pebble beds, a strong association between patterns in species diversity or assemblage structure and environmental features was observed at different spatial scales, suggesting that environmental control is an important process determining such patterns. This can be tested in future work by performing appropriate mensurative or manipulative studies (Underwood *et al*., 2000). Insights can also be obtained through contrasting simultaneous analyses of observational data using taxonomic and functional approaches (Anderson *et al*., 2011).

Implications for management

Characterisation of spatio-temporal patterns in species diversity and the structure of assemblages is a necessary precursor to formulating biologically sound management plans, particularly when these revolve around the establishment of marine protected areas (MPAs). For instance, representativeness is considered to be an important design criterion for MPAs (Dudley, 2008), but current Mediterranean habitat manuals only include the shallow pebble-bed assemblage. Thus MPAs designed on such incomplete knowledge could well include only these shallow habitats. This is counter-productive in two ways. Firstly, it fails to fulfil the aim of including examples of all the habitat types occurring in the region (Stevens, 2002). Secondly, this study has shown that among the three pebble-bed types, shallow pebble beds contain the lowest species richness with very few unique species, and therefore have the lowest conservation value.

Establishing a representative network of MPAs can only be achieved if habitat maps at the local scale of the MPAs are available. However, mapping exercises often rely heavily on abiotic variables to delineate habitat borders, implicitly assuming that abiotic variables correlate with patterns of biological distributions (Stevens and Connolly, 2004). It is therefore important to take a bottom-up approach to addressing representation, based on characterisation of the patterns in biological distributions and identification of surrogates that can be used to represent these patterns (Howell, 2010). Knowledge of the ecological mechanisms generating patterns is also essential for appropriate design of MPAs. For instance, if distributions are related to environmental conditions, reserves should represent each set of conditions together with favourable dispersal routes (Legendre *et al*., 2005).

In the present work, characterisation of infralittoral pebble-bed habitats revealed three distinct assemblage types, related to differences in environmental conditions. Thus, it is appropriate that all three pebble bed-types are represented within MPAs. The three variants were shown to be well represented by easily identifiable seascape factors. Consequently, any newly discovered pebble-bed patches can now be easily classified into ‘shallow’, ‘seagrass/creek’, or ‘harbour’ categories without the need for extensive sampling, facilitating benthic mapping exercises while also reducing the extent of destructive sampling. Meanwhile, the role of environmental control in maintaining a high beta diversity highlights the need for management plans to include measures that minimise the risk of adverse changes in environmental conditions. In this scenario any impact that causes homogenisation of conditions across sites would inevitably lead to an overall loss of species diversity (Balata *et al*., 2007).

Characterisation of infralittoral pebble beds has also provided the biological information required to assess their conservation value. The presence of pebble beds might have carried little weight in MPA site selection in the past, given that these habitats were generally considered to be impoverished (e.g. Bellan-Santini, 1985). On the other hand, this study has shown that these beds are rare habitats, at least in so far as total habitat area is concerned. They are also not impoverished, especially in the case of the deeper ‘seagrass/creek’ and ‘harbour’ types. Their conservation value will be even higher if it is shown that they contain species which do not occur elsewhere, but this can only be ascertained by comparison of the component species of different habitats. The discovery of three gobiid species previously unrecorded from the Maltese Islands and of new populations of the Maltese top-shell from the studied pebble beds suggests that this may well be the case, since none of these species are presently known to occur in other habitats. In fact, given that the Maltese top-shell is a Habitats Directive Annex II species, infralittoral pebble beds ought to be considered as priority habitats within the context of the Maltese Islands.

6.5 Conclusions

This study represents the first extensive characterisation of infralittoral pebble beds in the Maltese Islands, and probably also in the Mediterranean. Fifteen locations with accumulations of pebble beds large enough to study were located, but most of these covered relatively small areas (25–50 m^2) highlighting the overall rarity (in terms of total coverage) of these habitats.

Analyses of physical and biological attributes both indicated that three distinct pebble-bed types occur: shallow beds occurring in rocky coves, beds found within creeks or seagrass meadows, and beds located in harbour environments. Descriptions of the physical environment and characteristic species of each pebble-bed types have been produced. On the basis of these findings, suggestions for amendments to benthic habitat classification schemes have been proposed.

Overall, pebble-bed assemblages were diverse, with a total of 360 taxa being recorded from these habitats during the course of this study. Polychaetes, crustaceans and molluscs were the most common faunal groups. The recorded species included the endemic trochid gastropod, *Gibbula nivosa*, with three new populations being discovered. In addition, three gobiid species which had not previously been recorded from the central Mediterranean area were also found within the studied pebble beds.

Shallow beds were not as species rich as the other bed types; this could be due to the greater severity of physical disturbance. Indeed, a significant seasonal reduction in richness and total abundance was recorded from this assemblage, related to the higher magnitude of disturbance occurring during winter storms. On the other hand, the disturbance regime in deeper sites may increase diversity by preventing competitive exclusion, although this hypothesis needs to be confirmed by future work.

The high overall diversity was due to both a relatively high diversity per site, as well as between-site variation in species composition. The former is related to the small-scale structural complexity of pebble beds, which consequently provides a wide range of microhabitats and food sources for macrofauna. On the other hand, differences in the assemblage structure between sites and between pebble-bed types were associated with changes in the environmental conditions, indicating that environmental factors play an important role in determining spatial patterns of variation in the biotic assemblage.

Appendices

Appendix A

Classified list of taxa recorded during the present study, indicating the respective pebble-bed types from which each taxon was recorded. For details of the three bed types, see Chapters 2 and 3.

Some faunal groups were identified by experts as indicated in the 'Acknowledgments' section; for other fauna, identifications were mainly based on keys and descriptions in the following guides:

- Cachia, C., Mifsud, C. and Sammut, P.M., 1991. *The marine shelled Mollusca of the Maltese Islands (Part 1: Archaeogastropoda).* Grima Printing and Publishing Industries, Malta; 113pp.
- Cachia, C., Mifsud, C. and Sammut, P.M., 1996. *The marine Mollusca of the Maltese Islands (Part 2: Neotaenioglossa).* Backhuys Publishers, Leiden, Netherlands; 228pp.
- Cachia, C., Mifsud, C. and Sammut, P.M., 2001. *The marine Mollusca of the Maltese Islands (Part 3: Sub-class Prosobranchia to sub-class Pulmonata, order Basommatophora).* Backhuys Publishers, Leiden, Netherlands; 266pp.
- Cachia, C., Mifsud, C. and Sammut, P.M., 2006. *The marine Mollusca of the Maltese Islands (Part 4: The classes: Caudofoveata, Solenogastres, Bivalvia, Scaphopoda & Cephalopoda).* Backhuys Publishers, Leiden, Netherlands; 270pp.
- Falciai, L. and Minervini, R., 1992. *Guida dei Crostacei Decapodi d'Europa*. Franco Muzzio Editore, Padova, Italy; 282pp.
- Hayword, P.J. and Ryland, J.S. (eds.), 1995. *Handbook of the Marine Fauna of North-West Europe*. Oxford University Press, Oxford, UK; 800pp.
- Naylor, E., 1972. *Synopsis of the British Fauna No. 3: British Marine Isopods*. Academic Press Inc., London, UK; 90pp.
- Ruffo, S. (ed.), 1982. The Amphipoda of the Mediterranean, Part 1: Gammaridea (Acanthonotozomatidae to Gammaridae). *Mémoires de l'Institut Océanographique,* 13: 1–364.
- Ruffo, S. (ed.), 1989. The Amphipoda of the Mediterranean, Part 2: Gammaridea (Haustoriidae to Lysianassidae). *Mémoires de l'Institut Océanographique*, 13: 365–576.
- Ruffo, S. (ed.), 1993. The Amphipoda of the Mediterranean, Part 3: Gammaridea (Melphidippidae to Talitridae), Ingolfiellidea and Caprellidea. *Mémoires de l'Institute Océanographique*, 13: 577–813.

- Ruffo, S. (ed.), 1998. The Amphipoda of the Mediterranean: Part 4: Localities and map, Addenda to Parts 1-3, Key to Families, Ecology, Faunistics and Zoogeography, Bibliography, Index. *Mémoires de l'Institute Océanographique,* 13: 814–959.
- Saiz Salinas, J.I., 1993. *Fauna Iberica Vol. 4: Sipincula*. Museo Nacional de Ciencas Naturales, CSIC, Madrid, Spain; 200pp.
- Tortonese, E., 1965. *Fauna d'Italia VI: Echinodermata*. Edizioni Calderini, Bologna, Italy; 422pp.

Taxon	**Pebble-bed Type**		
	Shallow	**Seagrass / Creek**	**Harbour**
CNIDARIA			
Anthozoa			
Actiniaria sp.	✓	✓	✓
PLATYHELMINTHES			
Turbellaria			
Turbellaria Sp. A	✓	✓	✓
Rhabditophora			
?*Prosthiostomum* sp.	✓	✓	✓
Prostheceraeus giesbrechtii		✓	
NEMERTEA			
Nemertea sp. A		✓	✓
Nemertea sp. B	✓	✓	✓
Nemertea sp. C	✓	✓	✓
SIPUNCULA			
Aspidosiphon muelleri	✓	✓	✓
Phascolion strombus			✓
Phascolosoma granulatum	✓	✓	✓
Sipuncula sp. A			✓
ANNELIDA			
Polychaeta			
Ampharetidae sp.		✓	
Amphinomidae sp.	✓		✓
Aphrodita sp.	✓	✓	✓
Aphroditidae sp. A		✓	
Aphroditidae sp. B		✓	✓
Aphroditidae sp. C		✓	
Aphroditidae sp. D			✓
Capitellidae sp.	✓	✓	✓
Cirratulidae sp. A	✓	✓	✓
Cirratulidae sp. B	✓	✓	✓

Taxon	**Pebble-bed Type**		
	Shallow	**Seagrass / Creek**	**Harbour**
Cirratulidae sp. C	✓	✓	✓
Cirratulidae sp. D	✓	✓	✓
Cirratulidae sp. E		✓	✓
Cirratulidae sp. F			✓
Cirratulidae sp. G	✓	✓	✓
Cirratulidae sp. H		✓	✓
Dorvilleidae sp. A	✓	✓	✓
Dorvilleidae sp. B	✓	✓	✓
Dorvilleidae sp. C	✓		✓
Eunicidae sp. A		✓	✓
Eunicidae sp. B			✓
Eunicidae sp. C	✓	✓	✓
Eunicidae sp. D			✓
Eunicidae sp. E		✓	
Glycera sp.		✓	✓
Hermodice carunculata		✓	
Lepidonotus sp.		✓	✓
Lumbrineridae sp. A	✓	✓	✓
Lumbrineridae sp. B		✓	
Lysidice sp.		✓	
Maldanidae sp.	✓	✓	✓
Nematonereis unicornis	✓	✓	✓
Nephtyidae sp. A	✓	✓	✓
Nephtyidae sp. B	✓	✓	✓
Nephtyidae sp. C	✓	✓	✓
Nephtyidae sp. D			✓
Nereididae sp. A	✓	✓	✓
Nereididae sp. B	✓	✓	✓
Nereididae sp. C	✓		
Nereididae sp. D		✓	
Nereididae sp. E		✓	
Nereis rava	✓	✓	✓
Notomastus sp.	✓	✓	✓
Opheliidae sp. A	✓	✓	✓
Opheliidae sp. B	✓	✓	✓
Orbiniidae sp. A	✓	✓	✓
Orbiniidae sp. B	✓		
Paraonidae sp. A	✓	✓	✓
Paraonidae sp. B		✓	✓
Paraonidae sp. C		✓	✓
Paraonidae sp. D	✓	✓	✓
Paraonidae sp. E	✓		✓
Pectinariidae sp.	✓		✓
Pelogenia arenosa		✓	
Phyllodocidae sp. A		✓	✓
Phyllodocidae sp. B	✓	✓	✓

Taxon	**Pebble-bed Type**		
	Shallow	**Seagrass / Creek**	**Harbour**
Phyllodocidae sp. C	✓	✓	✓
Piromis eruca	✓	✓	✓
Polynoe sp. A	✓	✓	✓
Protodrilidae sp.	✓		
Sabellidae sp. A	✓	✓	✓
Sabellidae sp. B	✓	✓	✓
Sabellidae sp. C	✓	✓	✓
Sabellidae sp. D	✓	✓	✓
Sabellidae sp. E		✓	✓
Sabellidae sp. F		✓	✓
Serpulidae spp.	✓	✓	✓
Spionidae sp. A	✓	✓	
Spionidae sp. B	✓		
Spirorbinae spp.	✓	✓	✓
Sthenelais sp.		✓	✓
Syllidae sp. A	✓	✓	✓
Syllidae sp. B		✓	✓
Syllidae sp. C	✓	✓	✓
Syllidae sp. D	✓	✓	✓
Syllidae sp. E		✓	✓
Syllidae sp. F	✓		
Syllidae sp. G	✓		
Terebellidae sp. A	✓	✓	✓
Terebellidae sp. B	✓	✓	✓
Terebellidae sp. C		✓	✓
Terebellidae sp. D	✓	✓	✓
MOLLUSCA			
Polyplacophora			
Acanthochitona crinita	✓		
Acanthochitona fascicularis	✓	✓	✓
Callochiton calcatus			✓
Callochiton euplaeae	✓	✓	✓
Chiton (Rhyssoplax) corallinus		✓	
Chiton (Rhyssoplax) olivaceus	✓	✓	✓
Ischnochiton rissoi	✓	✓	✓
Lepidochitona cinerea	✓		
Lepidopleurus cajetanus			✓
Leptochiton sp. A		✓	
Leptochiton sp. B		✓	✓
Gastropoda			
Alvania beani			✓
Alvania discors	✓	✓	✓
Alvania lanciae		✓	✓
Alvania lineata	✓	✓	✓
Alvania mamillata	✓	✓	✓

Taxon	**Pebble-bed Type**		
	Shallow	**Seagrass / Creek**	**Harbour**
Alvania subcrenulata			✓
Bela menkhorsti		✓	
?Berthella sp.			✓
Bittium latreillii	✓	✓	✓
Bittium reticulatum	✓	✓	✓
Bittium submamillatum		✓	
Bulla striata		✓	✓
Caecum auriculatum			✓
Caecum ?clarki			✓
Caecum trachea			✓
Calliostoma conulus		✓	✓
Calliostoma laugieri			✓
Calliostoma zizyphinum			✓
Cerithium renovatum	✓	✓	
Cerithium vulgatum	✓	✓	✓
Cerithiopsis atalaya			✓
Cerithiopsis ?minima	✓		✓
Chrysallida ?interstincta		✓	
Clanculus corallinus			✓
Clanculus cruciatus		✓	✓
Clanculus jussieui	✓		✓
Clathromangelia granum		✓	✓
Columbella rustica	✓	✓	✓
Conus ventricosus	✓	✓	
Cumia reticulata		✓	
Dendrodoris sp.			✓
Diodora gibberula			✓
Eatonina cossurae	✓		
Elysia sp.	✓	✓	✓
Euspira intricata		✓	✓
Euthria corneum			✓
?Facelina sp.			✓
Fusinus rudis		✓	
Gibberula miliaria	✓	✓	
Gibberula philippii		✓	✓
Gibbula adansonii	✓	✓	
Gibbula ardens		✓	✓
Gibbula divaricata	✓		
Gibbula rarilineata	✓		
Gibbula fanulum		✓	✓
Gibbula guttadauri		✓	
Gibbula nivosa		✓	✓
Gibbula racketti	✓	✓	
Gibbula turbinoides			✓
Gibbula umbilicaris	✓	✓	✓
Gibbula varia	✓	✓	✓

Taxon	**Pebble-bed Type**		
	Shallow	**Seagrass / Creek**	**Harbour**
Granulina marginata		✓	
Haminoea hydatis	✓	✓	✓
Jujubinus exasperatus		✓	✓
Jujubinus gravinae		✓	
Jujubinus striatus		✓	✓
Mangelia multilineolata			✓
Mangelia taeniata		✓	
Mangelia unifasciata		✓	✓
Mangelia vauquelini		✓	✓
Melanella polita		✓	✓
Metaxia metaxia			✓
Mitra cornicula	✓	✓	
Mitrella scripta		✓	✓
Monophorus spp.			✓
Muricopsis cristata			✓
Nassarius cuvierii	✓	✓	✓
Naticarius hebraeus			✓
Odostomia conoidea		✓	✓
Ondina vitrea		✓	
Opisthobranchia sp. A		✓	✓
Opisthobranchia sp. B	✓	✓	
Parvioris ibizenca		✓	✓
Philine sp.		✓	
Philinopsis sp.		✓	✓
Phorcus richardi	✓		
Pisania striata	✓	✓	
Pisinna glabrata		✓	
Pusillina spp.	✓	✓	✓
Raphitoma laviae		✓	
Raphitoma philberti			✓
Rissoa sp. A	✓	✓	
Rissoa guerinii	✓	✓	
Rissoa variabilis		✓	
Rissoina bruguieri		✓	✓
Roxania utriculus		✓	
Setia sp.	✓	✓	
Similiphora similior			✓
Thuridilla hopei		✓	
Tornus subcarinatus	✓		
Tricolia pullus	✓	✓	
Truncatella subcylindrica	✓		
Turbonilla striatula		✓	
Vermetus granulatus	✓	✓	✓
Vermetus rugulosus		✓	
Vexillum ebenus		✓	✓
Vexillum savignyi		✓	✓

Taxon	**Pebble-bed Type**		
	Shallow	**Seagrass / Creek**	**Harbour**
Vexillum tricolor			✓
Vitreolina philippi		✓	✓
Weinkauffia turgidula		✓	
Williamia gussoni		✓	✓
Bivalvia			
Abra alba			✓
Abra ?segmentum		✓	
Anomia ephippium	✓	✓	✓
Arca noae		✓	✓
Arca tetragona			✓
Bornia sebetia	✓		
Cardita calyculata		✓	✓
Chama gryphoides		✓	✓
Ctena decussata		✓	✓
Dosinia exoleta			✓
Gari depressa			✓
Glans trapezia		✓	✓
Gouldia minima			✓
Hiatella arctica			✓
Irus irus		✓	✓
Limidae sp.		✓	
Lithophaga lithophaga			✓
Loripes lucinalis	✓	✓	✓
Modiolus barbatus			✓
Musculus costulatus		✓	
Nucula nitidosa		✓	✓
Ostrea edulis			✓
Papillicardium papillosum		✓	✓
Parvicardium exiguum		✓	
Parvicardium scriptum		✓	✓
Petricola lithophaga			✓
Pinctada imbricata radiata		✓	
Pitar rudis			✓
Polititapes aureus	✓	✓	✓
Pseudochama gryphina		✓	✓
Rocellaria dubia			✓
Striarca lactea			✓
Venus verrucosa			✓
ARTHROPODA			
Pycnogonida			
Pycnogonida sp.			✓
Ostracoda			
Ostracoda sp.	✓	✓	✓
Cirripedia			
Verruca sp.	✓		✓

Taxon	**Pebble-bed Type**		
	Shallow	**Seagrass / Creek**	**Harbour**
Leptrosraca			
Nebalia bipes	✓	✓	✓
Cumacea			
Bodotria ?arenosa	✓	✓	✓
Vaunthompsonia ?cristata	✓	✓	✓
Tanaidacea			
Apseudes talpa	✓	✓	✓
Leptochelia savignyi	✓	✓	✓
Tanais dulongi	✓	✓	✓
Mysidacea			
Siriella sp.	✓	✓	✓
Heteromysis sp.		✓	✓
Isopoda			
Astacilla sp.		✓	
Cleantis prismatica	✓		
Cyathura sp.	✓	✓	✓
Cymodoce truncata	✓	✓	✓
Dynamene spp.	✓	✓	✓
Eurydice sp.	✓		
Gnathia ?dentata	✓	✓	✓
Idotea sp.	✓		
Jaera sp.	✓		
Joeropsis sp.	✓	✓	✓
Janira sp.	✓	✓	
Munna sp.	✓		
Stenosoma lancifer	✓	✓	✓
Amphipoda			
Ampelisca ?ledoyeri		✓	
Amphilochus ?neapolitanus	✓	✓	
Aoridae sp.		✓	✓
Ampithoe ramondi	✓	✓	✓
Apherusa bispinosa		✓	
Atylus guttatus	✓	✓	
Atylus vedlomensis		✓	
Caprella acanthifera	✓	✓	✓
Caprella ?grandimana	✓	✓	✓
?Ceradocus sp.			✓
Cheirocratus sp. A	✓	✓	
Cheirocratus sundevalli	✓	✓	
Colomastix pusilla			✓
Deflexilodes acutipes		✓	✓
Dexamine spiniventris	✓		✓
Dexamine spinosa		✓	✓
Elasmopus brasiliensis	✓	✓	
Elasmopus pocillimanus		✓	
Ericthonius punctatus	✓	✓	

Taxon	**Pebble-bed Type**		
	Shallow	**Seagrass / Creek**	**Harbour**
Gammarella fucicola	✓	✓	✓
Hippomedon oculatus		✓	
Hyale camptonyx		✓	✓
Idunella nana		✓	✓
Jassa ocia	✓		
Lembos ?spiniventris			✓
Leptocheirus longimanus			✓
Leptocheirus pectinatus		✓	✓
Leucothoe ?spinicarpa		✓	✓
Lysianassa costae		✓	✓
Lysianassa pilicornis		✓	
Lysianassidae sp.	✓		
Maera grossimana	✓	✓	✓
Maera inaequipes	✓		
Maera pachytelson	✓		
Melita coroninii	✓	✓	
Melita hergensis	✓	✓	✓
Microdeutopus spp.	✓	✓	✓
Othomaera ?knudseni	✓		
Parhyale aquilina	✓	✓	
Pereionotus testudo	✓	✓	✓
Perioculodes longimanus	✓	✓	✓
Phtisica marina		✓	✓
Socarnes filicornis	✓	✓	
Stenothoe ?monoculodes	✓	✓	✓
Synchelidium ?longidigitatum	✓	✓	
Tryphosella ?minima	✓		
Decapoda			
Achaeus gracilis	✓	✓	✓
Alpheus dentipes		✓	✓
Alpheus macrocheles		✓	✓
Anapagurus sp.		✓	✓
Athanas nitescens	✓	✓	✓
Calcinus tubularis	✓	✓	✓
Callianassa sp.		✓	
Cestopagurus timidus	✓	✓	✓
Clibanarius erythropus	✓	✓	✓
Ebalia tumefacta		✓	
Ebalia edwardsii		✓	✓
Eualus cranchii		✓	✓
Galathea bolivari		✓	✓
Galathea intermedia		✓	✓
Hippolyte spp.	✓	✓	✓
Ilia nucleus		✓	✓
Pachygrapsus marmoratus	✓	✓	
Pagurus cuanensis			✓

Taxon	**Pebble-bed Type**		
	Shallow	**Seagrass / Creek**	**Harbour**
Parthenopoides massena	✓		✓
Pilumnus ?hirtellus			✓
Pisa ?tetraodon			✓
Pisidia bluteli	✓	✓	✓
Portunus hastatus		✓	
Processa sp.	✓	✓	✓
Sirpus zariquieyi		✓	✓
Upogebia tipica	✓	✓	✓
Upogebia deltaura			✓
Xanthidae sp.		✓	
Xantho pilipes	✓	✓	✓
ECHINODERMATA			
Ophiuroidea			
Amphipholis squamata	✓	✓	✓
Amphiura mediterranea		✓	
Ophioderma longicauda			✓
Ophiothrix ?fragilis			✓
Echinoidea			
Arbaciella elegans		✓	
Genocidaris maculata	✓	✓	✓
Paracentrotus lividus		✓	✓
Sphaerechinus granularis		✓	✓
Holothuroidea			
Holothuroidea sp.		✓	✓
Holothuria poli		✓	
Leptopentacta elongata		✓	
CHORDATA			
Ascidiacea			
Ascidiacea sp.		✓	✓
Ascidia sp.		✓	✓
Molgula sp.		✓	✓
Leptocardii			
Branchiostoma lanceolatum			✓
Actinopterygii			
Gobiidae spp.	✓	✓	✓
Gobius couchi			✓
Millerigobius macrocephalus			✓
Zebrus zebrus		✓	✓
Lepadogaster sp.	✓		✓

Appendix B

Mean (± SD) values of the physical parameters measured in the present study (see Chapter 2) for each of the nineteen sites. (n/a = no SD is available, since parameter was measured at the level of the site, not at the replicate level).

	Wied iz-Zurrieq		**Wied ix-Xoqqa A**		**Wied ix-Xoqqa B**		**Marsaxlokk**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Depth (m)	6.9	0.1	0.7	0.1	6.0	0.1	0.8	0.1
Area (m^2)	45	n/a	25	n/a	300	n/a	30	n/a
Thickness (cm)	5.50	0.58	6.00	1.63	3.75	0.50	8.75	1.50
Mean pebble size (mm)	32.8	5.4	26.6	8.4	39.6	9.7	57.3	23.0
Sand & Silt content (%)	7.2	14.5	3.4	5.9	21.3	3.9	8.1	8.0
Mean moment of inertia (g cm^2)	40	24	69	72	196	142	1511	2517
Total surface area (m^2)	0.83	0.05	0.73	0.23	0.34	0.03	0.71	0.42
Mean Elongation	0.71	0.02	0.73	0.02	0.73	0.02	0.73	0.03
Mean Flatness	0.65	0.07	0.61	0.02	0.67	0.03	0.70	0.04
Mean Roundness	0.63	0.04	0.71	0.02	0.65	0.03	0.64	0.02
Mean Sphericity	0.74	0.02	0.74	0.01	0.75	0.02	0.76	0.02
Filamentous algal cover (%)	3.58	4.83	5.38	7.54	6.08	2.10	0.00	0.00
Encrusting algal cover (%)	1.20	0.76	0.23	0.26	0.05	0.10	4.90	4.85
Ann. Av. Temperature (°C)	19.91	n/a	20.32	n/a	20.30	n/a	20.50	n/a
Ann. Av. Salinity (psu)	38.03	n/a	37.71	n/a	38.06	n/a	38.04	n/a
Ann. Av. Dissolved Oxygen (%)	109.4	n/a	93.4	n/a	101.8	n/a	102.6	n/a
Ann. Av. Turbidity (NTU)	0.36	n/a	0.62	n/a	0.12	n/a	0.43	n/a
Ann. Av. [Nitrate] (µg/L)	94.6	n/a	513.5	n/a	27.2	n/a	135.5	n/a
Ann. Av. [Phosphate] (µg/L)	7.0	n/a	4.6	n/a	1.8	n/a	2.1	n/a

	Hofra z-Zghira		**Ta’ Xbiex A**		**Ta’ Xbiex B**		**Manoel Island A**		**Manoel Island B**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Depth (m)	0.8	0.1	4.9	0.2	5.6	0.1	8.2	0.3	6.6	0.2
Area (m^2)	100	n/a	3000	n/a	3000	n/a	200	n/a	400	n/a
Thickness (cm)	5.00	0.00	5.00	0.82	5.50	1.00	4.25	0.50	4.50	0.58
Mean pebble size (mm)	21.2	2.3	25.5	4.1	21.1	3.8	33.4	6.9	24.6	3.2
Sand & Silt content (%)	8.2	5.7	38.0	7.6	38.8	1.5	40.8	7.7	38.9	6.8
Mean moment of inertia (g cm^2)	20	14	13	5	13	5	24	14	24	14
Total surface area (m^2)	0.85	0.18	0.50	0.17	0.50	0.17	0.40	0.06	0.40	0.06
Mean Elongation	0.75	0.01	0.71	0.02	0.71	0.02	0.76	0.03	0.76	0.03
Mean Flatness	0.56	0.03	0.63	0.04	0.63	0.04	0.72	0.13	0.72	0.13
Mean Roundness	0.74	0.01	0.72	0.01	0.72	0.01	0.73	0.01	0.73	0.01
Mean Sphericity	0.72	0.01	0.73	0.00	0.73	0.00	0.76	0.02	0.76	0.02
Filamentous algal cover (%)	6.85	4.74	9.85	3.17	9.85	3.17	13.88	2.75	13.88	2.75
Encrusting algal cover (%)	0.00	0.00	29.38	14.57	29.38	14.57	55.15	5.05	55.15	5.05
Ann. Av. Temperature (°C)	23.55	n/a	20.43	n/a	20.79	n/a	20.54	n/a	20.63	n/a
Ann. Av. Salinity (psu)	38.06	n/a	38.00	n/a	37.97	n/a	37.72	n/a	38.03	n/a
Ann. Av. Dissolved Oxygen (%)	107.3	n/a	110.2	n/a	111.6	n/a	111.9	n/a	107.6	n/a
Ann. Av. Turbidity (NTU)	0.33	n/a	1.12	n/a	0.38	n/a	0.34	n/a	0.65	n/a
Ann. Av. [Nitrate] (µg/L)	24.2	n/a	33.0	n/a	51.1	n/a	19.2	n/a	23.4	n/a
Ann. Av. [Phosphate] (µg/L)	1.8	n/a	1.7	n/a	1.9	n/a	1.7	n/a	1.8	n/a

	Tigné A		**Tigné B**		**Qawra**		**Mistra**		**Tunnara**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Depth (m)	7.5	0.3	10.1	0.3	1.4	0.1	1.2	0.1	1.0	0.0
Area (m^2)	3900	n/a	3900	n/a	40	n/a	100	n/a	30	n/a
Thickness (cm)	6.25	0.50	5.25	0.50	7.75	0.96	6.75	0.96	6.50	0.58
Mean pebble size (mm)	20.1	6.8	21.1	3.3	24.8	6.6	54.6	4.8	68.0	14.7
Sand & Silt content (%)	19.6	10.8	29.8	9.2	9.5	7.3	10.5	6.6	0.0	0.0
Mean moment of inertia (g cm^2)	22	10	22	10	26	15	368	133	1194	607
Total surface area (m^2)	0.40	0.11	0.40	0.11	1.01	0.23	0.44	0.01	0.51	0.15
Mean Elongation	0.75	0.01	0.75	0.01	0.74	0.01	0.77	0.01	0.75	0.01
Mean Flatness	0.63	0.03	0.63	0.03	0.63	0.02	0.71	0.01	0.64	0.02
Mean Roundness	0.71	0.04	0.71	0.04	0.70	0.01	0.67	0.04	0.74	0.02
Mean Sphericity	0.74	0.01	0.74	0.01	0.75	0.01	0.77	0.01	0.76	0.01
Filamentous algal cover (%)	8.95	3.96	8.95	3.96	0.00	0.00	4.33	2.04	3.93	2.53
Encrusting algal cover (%)	37.13	3.89	37.13	3.89	0.00	0.00	11.50	6.20	0.45	0.26
Ann. Av. Temperature (°C)	20.32	n/a	20.75	n/a	20.96	n/a	20.96	n/a	20.91	n/a
Ann. Av. Salinity (psu)	37.98	n/a	37.99	n/a	37.99	n/a	37.89	n/a	37.57	n/a
Ann. Av. Dissolved Oxygen (%)	105.3	n/a	105.6	n/a	108.5	n/a	99.1	n/a	113.7	n/a
Ann. Av. Turbidity (NTU)	0.49	n/a	0.34	n/a	0.17	n/a	2.66	n/a	0.64	n/a
Ann. Av. [Nitrate] (µg/L)	15.7	n/a	18.2	n/a	18.6	n/a	122.4	n/a	88.1	n/a
Ann. Av. [Phosphate] (µg/L)	1.8	n/a	2.3	n/a	2.3	n/a	2.9	n/a	3.1	n/a

	Hondoq ir-Rummien		Xatt l-Ahmar		Mgarr ix-Xini		Gnejna		Fomm ir-Rih	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Depth (m)	6.7	0.2	2.3	0.1	6.7	0.0	6.3	0.1	12.0	0.1
Area (m^2)	30	n/a	50	n/a	100	n/a	25	n/a	40	n/a
Thickness (cm)	3.50	0.58	2.00	0.00	3.00	0.00	6.50	1.73	4.50	1.29
Mean pebble size (mm)	28.2	5.4	19.3	4.1	12.8	3.0	38.2	5.9	14.5	2.4
Sand & Silt content (%)	23.1	11.4	13.2	3.2	17.4	4.9	14.7	11.1	16.9	2.9
Mean moment of inertia (g cm^2)	47	29	83	69	7	4	79	41	7	3
Total surface area (m^2)	0.47	0.11	0.32	0.12	0.38	0.05	0.59	0.15	0.53	0.23
Mean Elongation	0.74	0.02	0.76	0.01	0.75	0.03	0.74	0.01	0.73	0.04
Mean Flatness	0.70	0.02	0.67	0.04	0.68	0.03	0.68	0.03	0.62	0.03
Mean Roundness	0.59	0.01	0.65	0.04	0.60	0.02	0.69	0.01	0.73	0.01
Mean Sphericity	0.76	0.00	0.76	0.01	0.74	0.00	0.75	0.00	0.75	0.01
Filamentous algal cover (%)	3.53	4.17	13.05	3.56	2.13	1.43	5.15	3.82	3.53	1.55
Encrusting algal cover (%)	0.35	0.37	3.43	1.91	0.23	0.13	0.05	0.10	0.00	0.00
Ann. Av. Temperature (°C)	20.15	n/a	20.59	n/a	20.29	n/a	20.19	n/a	20.33	n/a
Ann. Av. Salinity (psu)	38.01	n/a	37.99	n/a	37.93	n/a	38.04	n/a	37.94	n/a
Ann. Av. Dissolved Oxygen (%)	108.8	n/a	110.6	n/a	107.3	n/a	111.1	n/a	111.7	n/a
Ann. Av. Turbidity (NTU)	0.13	n/a	0.65	n/a	0.33	n/a	0.11	n/a	0.05	n/a
Ann. Av. [Nitrate] (µg/L)	17.6	n/a	161.8	n/a	79.0	n/a	25.5	n/a	21.9	n/a
Ann. Av. [Phosphate] (µg/L)	2.1	n/a	2.3	n/a	2.3	n/a	2.6	n/a	2.5	n/a

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Publications

This section includes:

(i) the uncorrected author proofs of the following papers, which have been accepted for publication:

- Evans, J., Attrill, M.J., Borg, J.A., Cotton P.A. and Schembri, P.J. (in press). Macrofaunal diversity of infralittoral cobble beds in the Maltese Islands. To be published in *Rapport du Congrès de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée*, Vol. 40.
- Evans, J. and Schembri, P.J. (in press). The resurrection of *Gibbula nivosa* (Gastropoda: Trochidae). To be published in *Rapport du Congrès de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée*, Vol. 40.

(ii) the published version of the following paper:

- Kovačić, M., Bonello, J.J. and Evans, J. (2013). Three new records of Gobiidae from Malta with morphology, colouration and identification of the smallest known juveniles of two small gobiid species. *Cybium*, 37 (4): 233–239.

MACROFAUNAL DIVERSITY OF INFRALITTORAL COBBLE BEDS IN THE MALTESE ISLANDS

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Abstract

The Mediterranean "biocoenosis of infralittoral pebbles" has been poorly studied, but is generally considered to be impoverished. Systematic sampling of cobble beds at 17 sites around the Maltese Islands yielded a total of 35,687 individuals belonging to 310 different taxa. Very shallow sites (<2 m depth) had a slightly poorer faunal assemblage than deeper ones (2-12 m depth), but still included 152 taxa. These results suggest that infralittoral cobbles beds may not be as impoverished as previously thought, probably due to the high structural complexity of these habitats.

Keywords: Biodiversity, Infralittoral, Zoobenthos, Sicily Channel

Introduction

In shallow waters of the Mediterranean Sea, accumulations of pebbles and cobbles (particles between 4 mm – 256 mm) occurring in wave-exposed rocky coves support a distinct biotic community known as the "biocoenosis of infralittoral pebbles" [1]. No detailed ecological studies on this biocoenosis appear to have been carried out, but it is generally considered to be impoverished [2]. However, databases of Mediterranean marine fauna list "under stones" as the microhabitat of quite a number of species, and this habitat can be important recruitment ground for decapod Crustacea [3,4], indicating that infralittoral pebbles may be more species diverse than previously thought. In addition, coarse gravel sediments do not only occur in indentations along rocky coasts, but also in slightly deeper waters such as within inter-matte regions of reticulate *Posidonia oceanica* beds where a different suite of species could potentially be present. The present study was carried out to assess the macrofaunal diversity of infralittoral cobble beds in the Maltese Islands, in order to determine whether they are indeed impoverished habitats.

Material and Methods

Seventeen study sites having a continuous cover of pebbles and cobbles of at least 25 m^2 were selected from around the Maltese Islands; these included sites with cobble beds in both very shallow (<2 m) and deeper (2-12 m) waters. In 2011, four random samples were collected from each site by SCUBA divers. The pebbles within a 0.1 m^2 corer were carefully removed by hand and the basal layer of finer granules was scooped out separately. An air-lift suction sampler was simultaneously employed to reduce the risk of missing highly motile organisms, thus ensuring that quantitative samples of the total benthic fauna were collected. Samples were sorted in the laboratory and fauna retained by a 0.5 mm mesh were identified to the lowest possible taxon. Statistical analyses of the resulting species X site matrix were carried out using PRIMER v6 (Plymouth Routines In Multivariate Ecological Research, PRIMER-E Ltd.).

Results and Discussion

A total of 35,687 individuals, belonging to 310 separate taxa were recorded from a total sampling area of 6.8 m^2. The most common groups were Mollusca (118 taxa), Crustacea (89 taxa) and Polychaeta (74 taxa). Spirorbinae spp. accounted for 52.4% of all individuals and 90.3% of the polychaetes; thus Polychaeta was the most abundant faunal group overall, but non-spirorbid polychaetes comprised only 5.6% of the total fauna (Fig. 1). When sessile fauna are excluded, crustaceans were the dominant group; this agrees with studies of cobble sites in Norway, England and Italy [3]. The most ubiquitous species included the tanaid *Leptochelia savignyi*, the decapods *Athanas nitescens* and *Xantho pilipes*, numerous amphipods (of which *Ampithoe ramondi*, *Gammarella fucicola*, *Maera grossimana*, *Melita hergensis* and *Microdeutopus* spp. were the most abundant), the chiton *Ischnochiton rissoi*, the gastropod *Gibbula varia*, the polychaetes *Nereis rava*, Ophelidae spp. and Spirorbinae spp., and the ophiuroid *Amphipholis squamata*.

Cluster analysis indicated that the cobble bed assemblage composition varied with depth, with the very shallow (< 2m) sites generally grouping separately from the deeper ones (Fig. 2). The deeper sites (Cluster B) included 278 different taxa, versus the 152 taxa of 'Cluster A' sites, while 120 taxa were common to sites in both clusters. These results suggest that the biocoenosis of infralittoral cobbles and pebbles may not be as impoverished as previously thought, even if only the very shallow beds described in [1] are considered. This is probably due to the high structural complexity of the cobble habitats, which are characterised by vertical stratification: upper cobble layers can provide numerous interstitial spaces affording shelter to fauna [3,4] while the basal layer of finer sediment supports an infaunal assemblage.

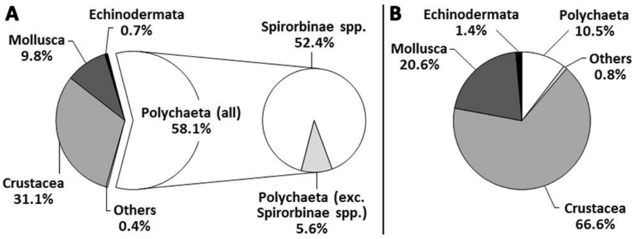

Fig. 1. Percentage abundance of major faunal groups from infralittoral cobble beds at 17 sites when considering (**A**) all macrofauna, and (**B**) non-sessile macrofauna only.

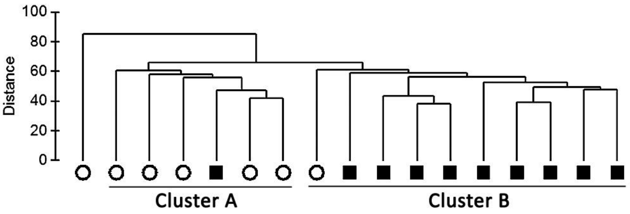

Fig. 2. Dendrogram from group-average hierarchical cluster analysis based on Bray-Curtis resemblances, produced using square root-transformed abundance data for macrofauna from infralittoral cobble beds at 17 sites (Empty circle: Depth <2 m; Filled square: Depth 2-12 m).

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THE RESURRECTION OF *GIBBULA NIVOSA* (GASTROPODA: TROCHIDAE)

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Abstract

The rediscovery of *Gibbula nivosa* from two widely separated cobble beds in 2006, after some 25 years during which no living specimens had been found, suggested that such beds may be an important habitat for this species. Systematic sampling of cobble patches at 15 different locations resulted in the discovery of another three populations, confirming the importance of cobble accumulations as a habitat for this species, but also showing that while certainly not extinct, *G. nivosa* is still quite rare within the Maltese Islands. This, together with its very narrow geographical range, being endemic to Malta, renders *G. nivosa* highly vulnerable to extinction.

Keywords: Biodiversity, Conservation, Endemism, Gastropods, Sicily Channel

Introduction

The Maltese top-shell, *Gibbula nivosa*, is a critically endangered marine trochid gastropod endemic to the Maltese Islands [1,2,3]. This species has always been rare and no live individuals were recorded between 1981 and 2006, despite intensive searches in localities where it used to occur [3]. In 2006, a population of *G. nivosa* was discovered within Sliema Creek, Marsamxett Harbour, while two individuals were also recorded off south-west Comino [1]. Although the main habitat of the Maltese top-shell has been considered to be leaves of the seagrass *Posidonia oceanica*, it has also been reported from under stones in shallow water [4], and the recently discovered populations were all recorded from such a habitat, indicating that cobble beds may be a more important habitat for *G. nivosa* than previously thought [1]. The present study was undertaken to assess the current status of the Maltese top-shell.

Material and Methods

A preliminary survey was carried out along the low-lying coasts of the Maltese Islands to map the presence of shallow-water 'cobble beds', defined as areas ≥ 25 m^2 having a continuous cover of pebbles and cobbles. Fifteen such beds were selected (Fig. 1), and four random samples were collected from each by SCUBA divers, using a 0.1-m^2 circular corer. The length and width of the sampled patched were also measured to estimate coverage. Samples were sorted in the laboratory, all molluscs present were identified, and any live individuals of *G. nivosa* were counted to obtain a measure of population density. Coverage and population density values were used to estimate the total population size within each of the sampled cobble beds with *G. nivosa*.

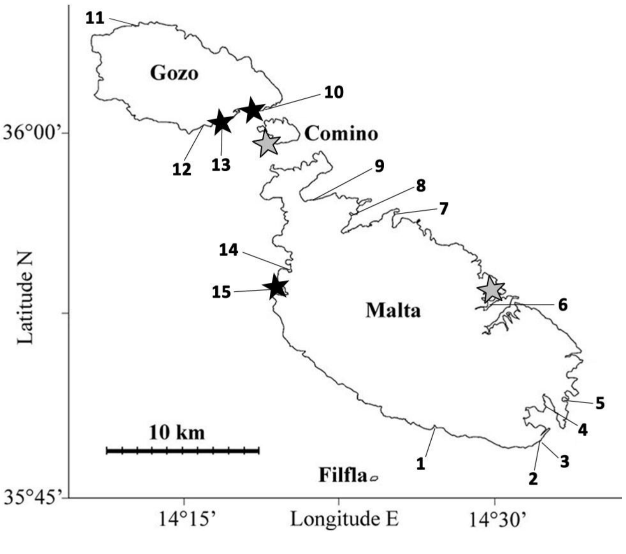

Fig. 1. Map of the Maltese Islands showing the location of the 15 sites with cobble/pebble habitats that where sampled (numbered 1-15); locations where live *Gibbula nivosa* populations were found in the present (black-filled stars) or recent [1,2] (grey-filled stars) studies are also indicated.

Results and Discussion

Live *G. nivosa* were recorded from three of the 15 sampled locations: Hondoq ir-Rummien (Site 10), Xatt l-Ahmar (Site 13) and Fomm ir-Rih (Site 15) (Fig. 1), with mean (±SD) densities of 2.0 ± 2.7, 0.5 ± 0.6 and 11.0 ± 13.4 ind./0.1 m^2, respectively. Given the restricted size of the cobble patches at the three sites, none of which exceeded 50 m^2, estimated population sizes were low, ranging between 250 and 4400 individuals. In the case of Hondoq ir-Rummien and Fomm ir-Rih, the bottom consisted of a sublittoral boulder scree adjacent to the coast, with *P. oceanica* meadows in deeper waters. Small patches of cobbles and pebbles were present as an enclave between these two habitats at depths of 7-12 m. Only the largest cobble patch was sampled at each site, and it is therefore possible that *G. nivosa* also occurred in cobble patches other than the ones sampled.

Following its rediscovery in 2006, *G. nivosa* has now been recorded from five separate locations around the Maltese Islands (Fig. 1), and other small populations may also exist in cobble patches around the Maltese coastline that have not been sampled. While never formally considered to be extinct, its reappearance after a 25-year period during which dedicated searches were unsuccessful [2] makes it a good example of a 'Lazarus species' [5]. All five presently known populations occur within accumulations of cobbles and pebbles, which therefore seem to be the main habitat for this species; past records from seagrass meadows may have resulted from collection of individuals during foraging excursions. This implies that past searches may have been unsuccessful because they were not made in the gastropod's primary habitat. Nevertheless, the present results also indicate that the Maltese top-shell is still a very rare species, being recorded from only three of the 15 sample sites, which, together with its very narrow geographical range (the Maltese Islands), renders it highly vulnerable to extinction.

Acknowledgements: Work on this protected species is in full conformity with the laws of Malta. We thank the Environment Protection Directorate of the Malta Environment and Planning Authority for issuing the necessary permits and for partial financial assistance; additional funding was provided through the European Social Fund under a STEPS scheme grant awarded to JE.

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Three new records of Gobiidae from Malta with morphology, colouration and identification of the smallest known juveniles of two small gobiid species

by

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Abstract. – Individuals of small juvenile gobies, some of them just 10 mm long, were collected during a study on infralittoral cobble bed fauna around the Maltese Islands between July and September 2011. These represent the first records of three gobiid species for Malta, including *Gobius couchi* Miller & El Tawil, 1974 and the smallest known juveniles of *Millerigobius macrocephalus* (Kolombatović, 1891) and *Zebrus zebrus* (Risso, 1827). Morphology and colouration, including diagnostic features and photographs, are provided for these species and the problems of identification of small juveniles are discussed.

Résumé. – Trois nouvelles signalisations de Gobiidae à Malte avec la morphologie, la coloration et l'identification des plus petits juvéniles connus de deux de ces petites espèces.

Des petits juvéniles de gobies, dont certains ne mesuraient que 10 mm, ont été collectés durant une étude de la faune des fonds de galets infralittoraux autour des îles maltaises entre juillet et septembre 2011. Ces derniers représentent pour Malte les premières signalisations de trois espèces de gobies: *Gobius couchi* Miller & El Tawil, 1974, les plus petits juvéniles connus de *Millerigobius macrocephalus* (Kolombatović, 1891) et de *Zebrus zebrus* (Risso, 1827). La morphologie et la coloration, comprenant les caractères utiles à la diagnose et les photographies, sont décrites pour ces espèces et les problèmes d'identification des petits juvéniles sont discutés.

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Key words
Gobiidae
Gobius couchi
Millerigobius macrocephalus
Zebrus zebrus
Mediterranean
Malta
Smallest juveniles
New records

Individuals of small juvenile gobies, some of them just about 10 mm long, were collected during a study on infralittoral cobble bed fauna around the Maltese Islands between July and September 2011. Gobies are small teleost fish generally under 100 mm of total length. They include the smallest marine fish species, and some of the smallest freshwater fish species belong to this group, with a mature size of about 10 mm or less (Nelson, 2006). In the Mediterranean the smallest known fish is a goby, *Speleogobius trigloides* Zander & Jelinek, 1976, which has a mature size of 24 mm, while 27% of Mediterranean gobiid species have a total length of less than 50 mm (Kovačić and Patzner, 2011). Identification of many Mediterranean gobiid species is difficult due to the small adult size and some morphological characters that are often difficult to see on small specimens, such as the head lateral line system. The morphology and colouration of gobiid juveniles can differ greatly from the adults, making identification of juveniles difficult (Kovačić, 2004). Papers with published descriptions or just illustrations of early juveniles for European marine gobiid species are rare and restricted to a few common species (summarized in Kovačić, 2004; Monteiro *et al*., 2008), and no data on early juveniles of the present species were available prior to this study.

The family Gobiidae *sensu* Nelson (2006) has the highest species richness among fish families in the Mediterranean, comprising more than 60 species with about 1/10 of all Mediterranean fish biodiversity (Quignard and Tomasini, 2000; Kovačić and Patzner, 2011). However, the patterns of actual species distribution of Mediterranean gobies are still unknown for most species, and the known diversity of Gobiidae along the Mediterranean coasts is continuously increasing, probably being far from the actual species richness (Kovačić and Patzner, 2011). Positive records of Gobiidae in Malta exist only for the ten species listed by Lanfranco (1993).

The aim of the present paper was to 1) provide data on juvenile morphology and colouration of the studied gobiid species, including the smallest known juveniles for two species, 2) discuss diagnostic features and identification of small gobiid juveniles, and 3) report their first records from Malta, based on specimens collected by two of the authors (JJB and JE) during a study on the biocoenosis of infralittoral cobble beds around the Maltese Islands, undertaken between July and September 2011.

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Cybium 2013, 37(4): 233-239.

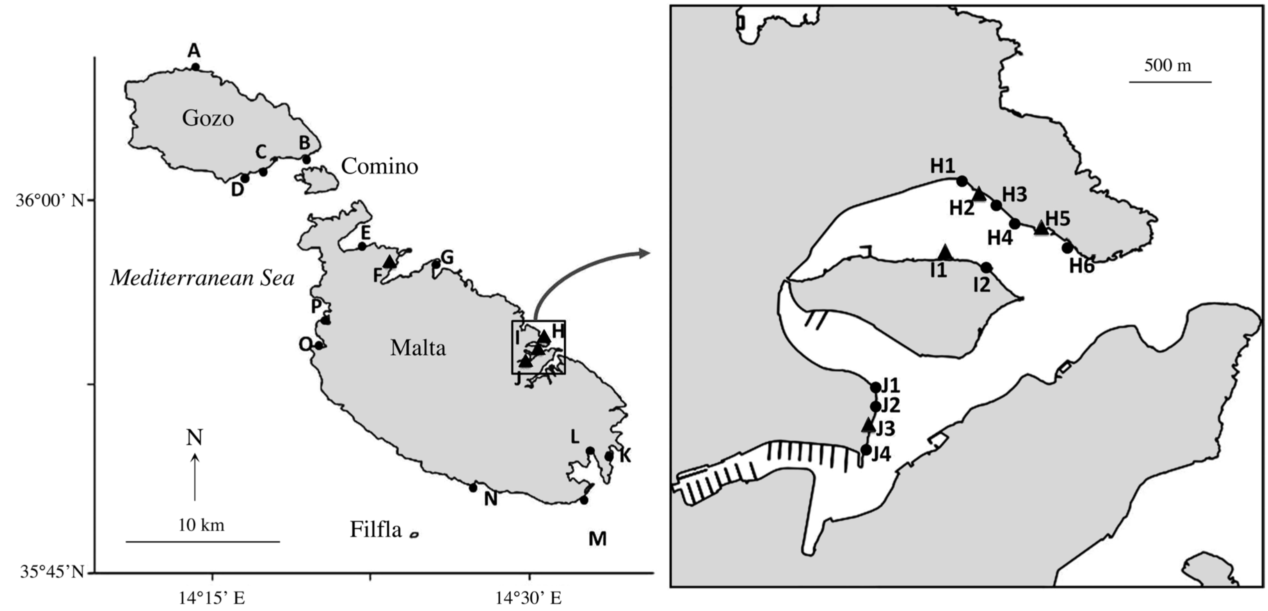

Figure 1. - Map of the Maltese Islands showing the sixteen sampling locations: (▲) sites with gobiid records; (●) sites from where no gobiid species were identified. *Key:* **A**: Wied l-Għasri; **B**: Ħondoq Bay; **C**: Ix-Xatt l-Aħmar; **D**: Mġarr ix-Xini; **E**: Mellieħa Bay; **F**: Mistra Bay; **G**: Qawra; **H1-H6**: Tigné A – Tigné F; **I1-I2**: Manoel Island A – Manoel Island B; **J1-J4**: Ta' Xbiex A – Ta' Xbiex D; **K**: Ħofra ż-Żgħira; **L**: Marsaxlokk; **M**: Wied ix-Xoqqa; **N**: Wied iż-Żurrieq; **O**: Fomm ir-Riħ Bay; **P**: Ġnejna Bay.

MATERIAL AND METHODS

A preliminary survey was carried out along the low-lying coasts of the Maltese Islands to map the presence of shallow-water 'cobble beds', defined as areas of not less than 25 m^2 having continuous cover of pebbles and cobbles. Cobble beds were present at sixteen locations (Fig. 1). A single sampling station was established at the centre of each location, except at Tigné, Manoel Island and Ta' Xbiex, where the presence of much larger beds (> 100 m^2) permitted use of multiple stations (see Fig. 1).

Four random samples of fauna were collected from each station using a 0.1-m^2 circular sampler (see Borg *et al.*, 2002) via SCUBA diving. Since the cobble beds were stratified, the top layer of cobbles and pebbles was carefully hand-picked and transferred to a 0.5-mm mesh bag, while a small fine-mesh hand net was used to scoop the basal layer of finer granules. A diver-operated air-lift suction sampler was simultaneously employed to reduce the risk of missing highly motile organisms, thus ensuring quantitative samples were collected. All fieldwork was carried out between July and September 2011.

Samples were subsequently sorted in the laboratory, with any Gobiidae present preserved in 70% ethanol and later identified by one of the authors (MK). The diagnoses presented are the minimum combination of characters that could identify the recorded species among gobiid species known in the CLOFNAM area (Miller, 1986; Ahnelt and Dorda, 2004; Kovačić, 2005 and references therein). Morphometric and meristic methods follow Schliewen and Kovačić (2008), while terminology of lateral-line system follows Sanzo (1911) and Miller (1986). All examined material has been deposited in the Natural History Museum Rijeka (PMR), Croatia.

RESULTS

Gobius couchi Miller & El-Tawil, 1974

Material examined

Juvenile of unidentified sex, PMR VP2884 (Fig. 2A), 18.2 + 4.2 mm, Tigné (H5), Malta, 29 Aug. 2011, coll. J.J. Bonello and J. Evans (Figs 1, 2B).

Diagnosis

(1) Suborbital papillae of lateral-line system without longitudinal row *a*; (2) all three head canals present; (3) scales present on predorsal area; (4) anterior oculoscapular canal with pore *α* at rear of orbit; (5) scales in lateral series on both sides 39 (known species range of scales in lateral series is 35-45, Miller, 1986); (6) pectoral fin 16 (known species range of pectoral fin rays 15-18, Miller, 1986); (7) row *d* divided below row *3*; (8) pelvic fin truncate (known species

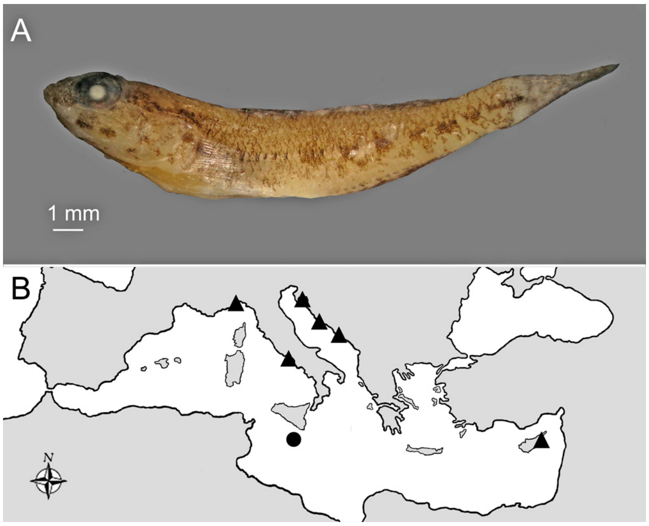

Figure 2. - *Gobius couchi*. **A**: Preserved specimen, PMR VP2884, juvenile of unidentified sex, 18.2 + 4.2 mm, Tigné E, Malta. **B**: Map of the Mediterranean showing previous records (▲) and new finding (●).

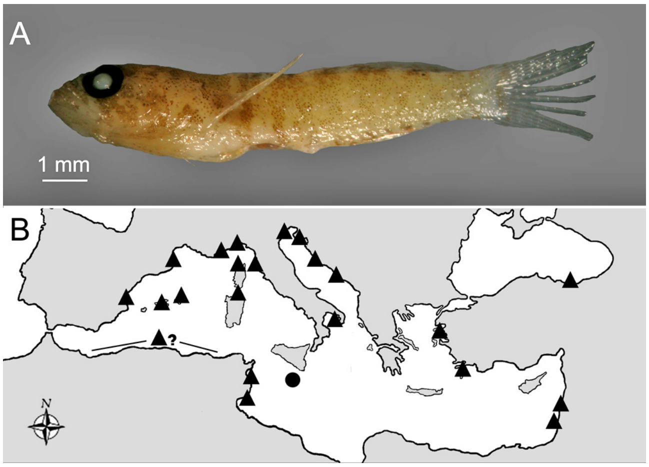

Figure 4. - *Zebrus zebrus*. **A**: Preserved specimen, PMR VP2878, juvenile of unidentified sex, 10.0 + 2.2 mm, Tigné B, Malta. **B**: Map of the Mediterranean showing previous records (▲) and new finding (●).

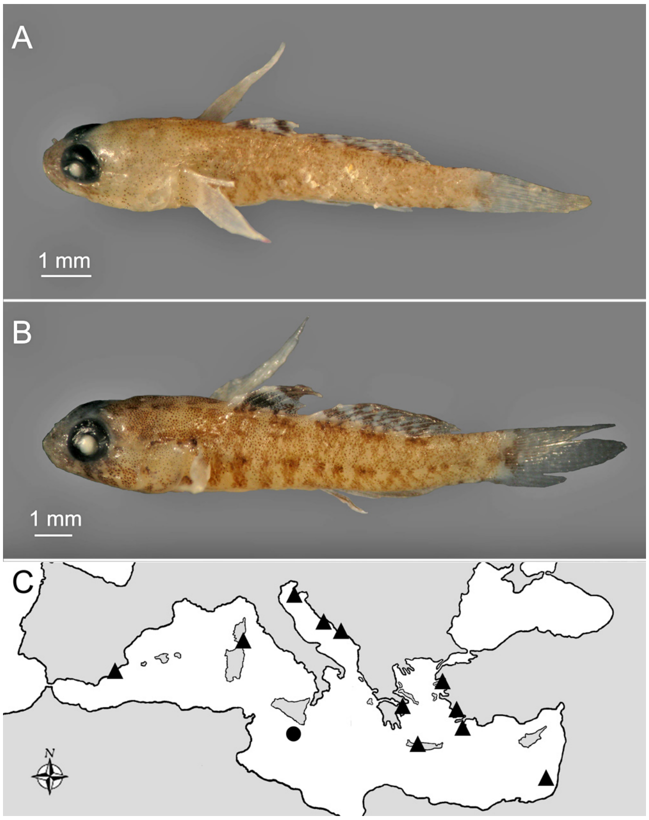

Figure 3. - *Millerigobius macrocephalus*. **A**: Preserved specimen, PMR VP2879, juvenile of unidentified sex, 8.5 + 2.4 mm, Manoel Island B, Malta. **B**: Preserved specimen, PMR VP2881, juvenile of unidentified sex, 11.5 + 2.9 mm, Manoel Island B, Malta. **C**: Map of the Mediterranean showing previous records (▲) and new finding (●).

with rounded to truncated pelvic fins, Miller, 1986).

Description

Anterior nostril short, tubular, with higher posterior rim. Branchiostegal membrane attached to entire side of isthmus. First dorsal fin VI, second dorsal fin I/13, anal fin I/12, caudal fin with 14 branched rays and 16 segmented rays, pectoral fin 16, pelvic fins I/5+5/I. Free tips on pectoral fin rays not visible. Pelvic fin truncate. Anterior membrane height in midline 1/3 length of pelvic fin's spinous ray. Body with ctenoid scales, scales in lateral series 39. Opercle and cheek naked, scales present on predorsal area. Preserved colour: body light brown with reticulate pattern, formed by pigmentation along the margins of most scales. Breast and belly pale. Most distinct marks were about nine darker blotches along lateral midline. Head similar to body, with two dark spots on cheek and dark-pigmented snout. Three longitudinally arranged dark gular spots with fourth dark spot on ventral part of opercle. Dorsal and caudal fins with rows of small dark dots. Anal fin and the bases of anal fin rays pigmented. Pectoral fin with dark mark in upper fin origin, mark deeper than long, another dark mark nearby on upper pectoral base. Pelvic fins pale. Head with anterior and posterior oculoscapular, and preopercular canals, with pores $\sigma, \lambda, \varkappa, \omega, \alpha, \beta, \varrho, \varrho^1, \varrho^2$, and $\gamma, \delta, \varepsilon$, respectively. Rows of suborbital papillae of lateral-line system without longitudinal row *a;* six transverse suborbital rows of sensory papillae. Longitudinal suborbital row *d* divided below suborbital row *3*. Oculoscapular anterior longitudinal row x^1 ending anteriorly behind pore β.

Geographical and ecological data

This single juvenile was recorded from a depth of 10 m at Tigné (H5) (the north-east coast of Malta Island), where the habitat was characterised by a gently sloping bottom of gravelly sand and silt with overlying accumulations of cobbles and pebbles that were generally encrusted by coralline algae. The specimen was collected from within the cobble layer.

Remarks

The morphology of the juvenile was developed enough to match diagnostic characters of adults of the species. Visible scales were present on the predorsal area, but the area was not completely scaled. Šanda and Kovačić (2009) reported on the complete absence of scales at the predorsal area in a small juvenile (15.6 + 4.1 mm), but present in a larger specimen of 19.5 + 5.0 mm. The basic adult colouration pattern could be recognised on the juvenile from Malta.

G. couchi is a goby known from Atlantic localities in Great Britain and Ireland and from several localities in the north and eastern Mediterranean (Kovačić *et al.*, 2012). The present finding at Malta represents a south-east extension of the known range of this species (Fig. 2B).

Millerigobius macrocephalus (Kolombatović, 1891)

Material examined

Juvenile of unidentified sex, PMR VP2879, 8.5 + 2.4 mm, Manoel Island (I1), Malta, 31 Aug. 2011, coll. J.J. Bonello and J. Evans (Fig. 3A); juvenile of unidentified sex, PMR VP2880, 11.4 + 2.8 mm, Manoel Island (I1), Malta, 31 Aug. 2011, coll. J.J. Bonello and J. Evans; juvenile of unidentified sex, PMR VP2881, 11.5 + 2.9 mm, Manoel Island (I1), Malta, 31 Aug. 2011, coll. J.J. Bonello and J. Evans (Fig. 3B); juvenile of unidentified sex, PMR VP2883, 10.2 + 2.6 mm, Tigné (H2), Malta, 23 Aug. 2011, coll. J.J. Bonello and J. Evans; (Figs 1, 3C).

Diagnosis

(1) Suborbital papillae of lateral-line system without longitudinal row *a*; (2) anterior oculoscapular and preopercular canals present, posterior oculoscapular canal absent; (3) pelvic fins forming disc; (4) interorbital papillae present.

Description

Anterior nostril tubular, long, reaching upper lip, without process from rim. Posterior nostril slightly raised. Branchiostegal membrane attached to entire side of isthmus. First dorsal fin VI; second dorsal fin I/10; anal fin I/9; caudal fin with 12-13 branched rays and 16-17 segmented rays; pectoral fin 15-16; pelvic fins I/5+I/5. Pelvic fins forming disc, with anterior transverse membrane. Body with ctenoid scales, scales in lateral series 28-31. Head, predorsal area and breast naked. Preserved colour: Specimen PMR VP2881 (Fig. 3B), 11.5 + 2.9 mm with body yellowish brown. About seven vertical dark brown bands on the body, darkest anteriorly, posteriorly, bands lighter and blurred, present along lateral side below dorsal fins. About nine dark lateral midline spots present at vertical bands, posteriorly on caudal peduncle bands no longer visible. Reticulate pattern, formed by dark markings along the scale margins, visible. Pectoral fin base, isthmus and breast pale compared to rest of body, but pigmented. Belly unpigmented. Head unequally pigmented, with palest part behind eye at level of pupil and on opercle. Specimens PMR VP2880, 11.4 + 2.8 mm, PMR VP2883, 10.2 + 2.6 mm and PMR VP2879 (Fig. 3A), 8.5 + 2.4 mm with body greyish brown, dominated by grey-brown melanophores. Vertical dark bands and dark lateral midline spots on the body indistinct. Reticulate pattern, formed by dark markings along scale margins, visible in PMR VP2880, 11.4 + 2.8 mm, but still not developed in specimens PMR VP2883, 10.2 + 2.6 mm and PMR VP2879, 8.5 + 2.4 mm. Pectoral fin base, isthmus, breast and belly pale compared to rest of body, unpigmented in PMR VP2879, 8.5 + 2.4 mm, and pigmented in specimen PMR VP2880, 11.4 + 2.8 mm. Head colouration pattern also dominated by densely scattered grey-brown melanophores. Fin colouration similar in all specimens. First dorsal fin with one transparent longitudinal band at middle of fin and two broad dark bands above and below it. Second dorsal fin with irregularly scattered bright dots; in smallest specimen PMR VP2879, 8.5 + 2.4 mm with two dark longitudinal bands. Dark brown 3-shaped mark present on origin of caudal fin, reaching caudal peduncle at midline and leaving pale areas at upper and lower caudal fin base, indistinct in smallest specimen PMR VP2879, 8.5 + 2.4 mm. Anal fin pigmented. Pectoral and pelvic fins pale. Head with anterior and preopercular canals, with pores *σ, λ, ϰ, ω, α, β, ϱ* and *γ, δ, ε* respectively, except in specimen PMR VP2879, 8.5 + 2.4 mm where parts of both canals are present as open furrows. Rows of sensory papillae: No suborbital row *a*. Seven transverse suborbital rows of sensory papillae. Row *b* anteriorly beginning below rear border of eye. Two or four interorbital papillae present behind pore *λ*.

Geographical and ecological data

Four individuals of *M. macrocephalus* were collected in all, three from Manoel Island (I1) (depth: 6 m) and one from Tigné (H2) (depth: 10 m), both localities placed at the north-east coast of Malta Island. All four specimens were found within a cobble habitat similar to that found at Tigné (H5), described above.

Remarks

The morphology of all four juveniles was developed enough to match diagnostic characters of the species in adults. The basic colouration pattern of the adults could be recognised on the juvenile PMR VP2881, 11.5 + 2.9 mm (Fig. 3B), but was still not developed in three other early juveniles (specimens PMR VP2880, 11.4 + 2.8 mm, PMR VP2883, 10.2 + 2.6 mm and PMR VP2879, 8.5 + 2.4 mm, (Fig. 3A). The present findings are the smallest known specimens of this species. All previously published records of *M. macrocephalus* (Fig. 3C) were of significantly larger males or females, except in Kovačić *et al*. (2011), who reported slightly larger juveniles (of unidentified sex, 12.1 + 2.9 mm and 12.2 + 3.1 mm) with the diagnostic characters of *M. macrocephalus* and with a photo of a smaller specimen with recognisable adult colouration pattern (Fig. 8 in Kovačić *et al*., 2011). *M. macrocephalus* is a small Mediterranean cryptobenthic gobiid species known from Mar Menor (Spain) in the west, along the north coast of the Mediterranean to the Levant (Israel) in the east (Kovačić *et al*., 2012). The present finding at Malta represents a south-east extension of the known range of this species (Fig. 3C).

*Zebrus zebrus (*Risso, 1827)

Material examined

Juvenile of unidentified sex, PMR VP2878 (Fig. 4A), 10.0 + 2.2 mm, Ta' Xbiex (J3), Malta, 15 Aug. 2011, coll. J.J. Bonello and J. Evans; juvenile of unidentified sex, PMR VP2882, 9.4 + 2.3 mm, Mistra, Malta, 28 Jul. 2011, coll. J.J. Bonello and J. Evans; (Figs 1, 4B).

Comparative material of *Z. zebrus*

One juvenile of unidentified sex, 11.9 + 3.5 mm, PMR VP2778, Kupari, Dubrovnik, southern Adriatic Sea (42°37'10.7''N; 18°11'28.8''E), 14 Sep. 2011, coll. M. Kovačić, M. Kirinčić and D. Zanella; 1 juvenile of unidentified sex, 10.4 + 2.7 mm, PMR VP2779, beach between Slano and Trsteno, Dubrovnik, southern Adriatic Sea (42°37'10.7''N; 18°11'28.8''E), 15 Sep. 2011, coll. M. Kovačić, M. Kirinčić and D. Zanella.

Diagnosis

(1) Suborbital papillae of lateral-line system without longitudinal row *a*; (2) predorsal area naked; (3) transverse suborbital rows 7; 4) pelvic fins forming disc; 5) interorbital papillae absent; (6) scales in lateral series 29-30 (the known species range of scales in lateral series is 29-38, Miller, 1986).

Description

Anterior nostril short, tubular, no visible tentacle from inner part of rim. Branchiostegal membrane attached to entire side of isthmus. First dorsal fin VI; second dorsal fin I/11; anal fin I/9; caudal fin with 13 branched rays, 16 segmented rays; pectoral fin 17; pelvic fin I/5+I/5. Uppermost rays of pectoral fin still not free from membrane. Pelvic fins forming disc. Body with ctenoid scales, scales in lateral series 29-30. Head and predorsal area naked. Colour preserved: body yellowish brown, brown melanophores on the body arranged in vertical bands, darker and more intensive on anterior part of body, paler posteriorly (Fig. 4A). Vertical bands more visible in the larger PMR VP2878 10.0 + 2.2 mm and less distinguishable in the smaller PMR VP2882 9.4 + 2.3 mm. Breast and belly pigmented. Head pigmented with brown melanophores, underside whitish, but with melanophores. Predorsal area densely pigmented. Dorsal and anal fins pigmented, fin membranes too damaged to determine colouration pattern. Caudal fin lightly pigmented, with brown vertical band present on the origin of caudal fin. Pectoral fin pigmented on upper bases of rays, rest of fin colourless. Pectoral fin base pigmented, more intensive dorsally. Pelvic fins colourless. Head with anterior oculoscapular and preopercular canals, with pores $\sigma, \lambda, \varkappa, \omega, \alpha, \beta, \varrho,$ and $\gamma, \delta, \varepsilon,$ respectively. Posterior oculoscapular canal still not developed or present only as an open furrow. Rows of sensory papillae: No interorbital rows. No suborbital row *a*. Seven transverse suborbital rows.

Geographical and ecological data

This species was recorded from within cobbles at two sites, Ta Xbiex (J3) and Mistra, both located at the north-east coast of Malta Island. The former site is characterised by a habitat similar to that found at Tigné (H5), described above, and the specimen was collected from a depth of 5 m. The habitat at Mistra consisted of patches of cobbles and pebbles found interspersed with *Posidonia oceanica* beds at shallow depths of 1-2 m.

Remarks

The presently recorded juveniles are the smallest known specimens of this species. The smallest juvenile (14.5 + 3.7 mm) already having recognisable adult colouration pattern and morphology was reported by Kovačić and Engin (2009), with a photo of the specimen included as their Fig. 2. Contrary to that record, the present juvenile specimens are without posterior oculoscapular head canal or free tips of uppermost pectoral rays and tentacle on the anterior nostrils. In the comparative material of *Z. zebrus* (10.4 + 2.7 mm, PMR VP2779 and 11.9 + 3.5 mm, PMR VP2778), the posterior oculoscapular canal is still not developed in the smaller specimen (10.4 + 2.7 mm, PMR VP2779), but it is visible in the larger one; both specimens have free tips to the uppermost pectoral rays, but both lack the tentacle on the anterior nostrils. The body colouration pattern of the present juvenile specimens with vertical bands resembles the known pattern of adults (Fig. 4A). Nevertheless, to ensure positive species identification of the present juvenile specimens, the following additional characters were added to the diagnosis to distinguish these specimens from the known Mediterranean gobiid species having anterior oculoscapular and preopercular canals present and lacking the posterior oculoscapular canal: pelvic fins forming disc *vs*. pelvic fins almost separate in *O. balearica* and *Vanneaugobius* species; interorbital papillae absent *vs*. interorbital papillae present in *M. macrocephalus*; 7 transverse suborbital rows *vs*. 6 transverse suborbital rows in *Didogobius schlieweni* and *D. splechtnai*; scales in lateral series 29-30 in the present material vs. scales in lateral series more than 41 for *Chromogobius zebratus* and more than 56 for *C. quadrivittatus*. *Z. zebrus* is a small cryptobenthic goby widespread in the Mediterranean and also recorded in the Black Sea (Kovačić *et al*., 2012). The present record of *Z. zebrus* from Malta connects the previously known distributions of this species in Western and Eastern Mediterranean (Fig. 4B).

DISCUSSION

Mediterranean gobies have high species diversity (Quignard and Tomasini, 2000; Kovačić and Patzner, 2011). However, significant proportions of these fish species are of small size, and many gobies are exclusively or predominantly of cryptobenthic occurrence. Thus many of them are still poorly known and, until recently, considered to be extremely rare (Patzner, 1999; Kovačić and Patzner, 2011). *G. couchi* was for the first time recorded in the Mediterranean in 1999 (Stefanni and Mazzoldi, 1999) and the known number of records of this species and *M. macrocephalus* is still limited (Figs 2B, 3C). The collection of small cryptobenthic fishes requires special methods, differing from the usual collecting gear for marine fishes. The use of SCUBA diving combined with use of anaesthetic, handnets, suction samplers and careful checks of small hidden habitats should be suitable to collect these fishes (Patzner, 1999). The methods used in the study on infralittoral cobble beds fauna of the present research enabled this collection of very small juvenile gobies, some of them just 10 mm long, in cryptobenthic habitats.

Since the morphology of gobiid juveniles can differ greatly from that of the adult stages (Kovačić, 2004), gobiid identification keys that make use of adult characters are not applicable to specimens that have not yet reached a certain size or developmental stage. This issue was not discussed in the identification key for European marine gobies by Miller (1986), but it was noticed as a problem in the identification key for Adriatic gobies by Kovačić (2008). Kovačić (2008) stated that his key could be used to identify adults of both sexes as well as late juveniles of all Adriatic gobiid species, but not the early juveniles that have not yet completely developed the characters used in the key. In addition, no published keys or diagnoses exist for the early juveniles of European marine gobies (Kovačić, 2004). Papers with published descriptions or illustrations of early juveniles of European marine gobiid species are rare (summarized in Kovačić, 2004; Monteiro *et al*., 2008). These data, restricted to a few common species, cannot be used for identification of early juveniles of numerous European marine gobiid species. Published keys and diagnostic characters for larvae (Lebour, 1919; Borges *et al*., 2003) are also restricted to a limited number of species, and make use of a combination of vertebral and fin counts and larval pigmentation which are not applicable to juveniles. Therefore, even in gobiid species where larvae can be identified, an identification gap exists at the early juvenile stages. To confirm morphological results, Monteiro *et al*. (2008) and Šanda and Kovačić (2009) validated the identification of early stages by comparison of DNA sequences with the sequences of positively identified adults.

The present findings with the small (*G. couchi*) or even the smallest known specimens for studied species (*M. macrocephalus*, *Z. zebrus*) offered the chance to check the morphology and identification methods at these specimen lengths. In the case of *G. couchi*, the present specimen showed that juveniles at standard length of about 18 mm and total length of about 22 mm can be identified by the species diagnoses and published identification keys (Miller, 1986; Kovačić, 2008). However, at a standard length of about 16 mm and total length of about 20 mm, the complete absence of visible scales on the predorsal area required a different approach for species identification (Šanda and Kovačić, 2009). *M. macrocephalus* could be identified by species diagnoses and published keys for identifications (Miller, 1986; Kovačić, 2008) at standard length of just about 9 mm and total length of just 11 mm. However, at this size the specimen still had early juvenile colouration, which differs from the later colouration pattern observed in specimens having standard length of about 12 mm and total length of about 14 mm. *Z. zebrus* could be identified by published species diagnoses or identification keys (Miller, 1986; Kovačić, 2008) at a standard length of about 12 mm and total length of about 15 mm, even though the completely developed morphology is attained later in development, at standard length of about 15 mm and total length of about 18 mm (Kovačić and Engin, 2009). However, at standard length of about 11 mm and total length of about 13 mm or smaller, the posterior oculoscapular canal is still not developed and a different approach is required for species identification of specimens at this or smaller lengths. Early juvenile colouration (with vertical bands) at the smallest recorded size of standard length of about 10 mm and total length of about 12 mm already resembles the adult colouration. Knowledge of the minimum size threshold that must be reached to enable positive species identification would help the process of identification of Mediterranean gobiids which, for many species, is difficult enough for adults. However, different intraspecific growth rates of juveniles could pose a problem for using such minimum length limits since there is no published knowledge on growth rates and the early development of morphological characters.

In terms of biogeography, Malta has been placed in different marine biogeographical sectors depending on the organisms being taken into consideration (Bianchi, 2007 and references therein) as it lies at or close to the meeting point between three bioregions: the Tyrrhenian Sea to the north west, the upper Ionian Sea to the north east, and the Gulf of Gabès to Levant Sea area to the south and south east (Bianchi and Morri, 2000; Bianchi, 2007). For the Gobiidae, Kovačić and Patzner (2011) set the border between the northern Mediterranean area rich with gobies (> 40 species) and the species-poor middle southern part of the Mediterranean (12 species) to the north of Malta; therefore Malta was somewhat arbitrarily placed in the middle south part of the Mediterranean. Only ten previously known gobiid species with positive records in Malta (Lanfranco, 1993) support this assignment of Malta into the species-poor middle south part of the Mediterranean by Kovačić and Patzner (2011). However, the present records have extended the south-eastern geographic distribution for *G. couchi* and *M. macrocephalus*. These findings of rarely recorded species (Figs 2B, 3C), restricted until now to the north Mediterranean and the Levant, suggest that the gobiid diversity of Malta could be rich and similar in composition to the northern Mediterranean areas noted by Kovačić and Patzner (2011). Despite this, only additional systematic sampling for all gobiid species in various habitats around the Maltese Islands will provide data for a check-list of gobies from Malta and answer the question concerning the relationship of Maltese gobiid fauna with that of the surrounding mainland coasts. Furthermore, while the present records of rarely recorded species suggest that the Maltese gobiid fauna could be similar to that of the north Mediterranean, the southwards extension of the known range of some species indicates that their distribution is more widespread than previously thought. This suggests that they might also be found from the middle-south part of the Mediterranean if proper sampling for such cryptobenthic species is undertaken.

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