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1 **Hidden in plain sight: species richness and habitat characterisation of sublittoral pebble**  
2 **beds**

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15

16 **Abstract**

17 Sublittoral pebble beds are generally considered to be impoverished, but the physical and

18 biological characteristics of these habitats are poorly known. We characterised nineteen

19 pebble bed sites in the Maltese Islands, providing detailed habitat data for Mediterranean

20 sublittoral pebble beds for the first time. Nearly 40,000 individuals belonging to 332 taxa

21 were recorded in all, with total richness estimated to reach 440 taxa; molluscs, crustaceans

22 and polychaetes were the most diverse faunal groups. This high diversity is likely due to the

23 structural complexity of the pebble beds, which had a vertically stratified arrangement of

24 sediment particles that is likely maintained through periodic physical disturbance. Variation

25 in the biotic assemblage from site to site was correlated with changes in the quantity of sand

26 and silt, with the area of the pebble bed, with water depth, and with the thickness of the  
27 pebble layer. This indicates that pebble-bed macrofaunal assemblages are sensitive to  
28 changes in hydrodynamic conditions and sediment loading, to alterations to the stratification  
29 of the pebble beds, and to fragmentation of the habitat patches. These results contradict  
30 assertions that sublittoral pebble beds are impoverished, instead showing that they can be  
31 highly diverse habitats supporting biotic assemblages that respond to a complex set of  
32 environmental variables. The present findings enable better understanding of the ecological  
33 importance of pebble beds and of the potential impacts that may be caused by anthropogenic  
34 disturbance, thereby enabling more informed decisions for habitat conservation and  
35 management.

36

37 **Keywords:** Biodiversity, Conservation, Cobble bed, Community composition, Habitat,  
38 Malta, Mediterranean

39

40

## 41 **Introduction**

42

43 The 1992 Rio de Janeiro Convention on Biological Diversity heralded the “era of  
44 biodiversity” (Boero 2010) where exploration of diversity patterns and their environmental  
45 correlates became more imperative than ever. Nonetheless, to date the spatial patterns of  
46 marine benthic diversity remain poorly known for many habitats (Costello et al. 2010).  
47 Sublittoral cobble and pebble beds are a case in point. Quantitative descriptions of cobble or  
48 pebble biota are rare, possibly because conventional sampling methods such as cores or grabs  
49 are not suitable for these habitats (Linnane et al. 2003). We are aware of only five studies  
50 published in the mainstream literature that looked at the entire macrofaunal assemblage of

51 these habitats, based on sites in Canada, England, Ireland, France (Atlantic coast) and  
52 Norway (Scheibling and Raymond 1990; George et al. 1995; Linnane et al. 2003; Ringvold et  
53 al. 2015; Foveau and Dauvin in press). The few other studies that have been undertaken on  
54 these habitats focused exclusively on specific groups such as macrophytes (Lieberman et al.  
55 1979, 1984; Davis and Wilce 1987a, 1987b; Scheibling et al. 2009a, 2009b), sessile biota  
56 (Osman 1977; Balazy & Kuklinski 2003, 2007), or decapods (Robinson and Tully 2000a,  
57 2000b; Linnane et al. 2001). Of these, only Robinson and Tully (2000a) investigated the  
58 spatial variability in community structure in relation to substratum characteristics, but they  
59 also included areas that consisted predominantly of coarse sand or bedrock amongst their five  
60 sites.

61

62 Consequently not much is known about the physical or biological characteristics of sublittoral  
63 pebble and cobble habitats. These habitats are generally considered to be faunally  
64 impoverished (Connor et al. 2004; Foveau and Dauvin in press), a view possibly extrapolated  
65 from intertidal cobble and pebble habitats which have been described as “intertidal deserts”  
66 where macrobiota are absent (Little 2000). Accordingly, habitat classification systems used  
67 for marine conservation and management assume that there is little of interest in ecosystems  
68 comprising pebbles and cobbles. Yet this view does not appear to have been borne out by  
69 empirical studies, since the limited information available indicates that these habitats may not  
70 be so depauperate. For instance, between 15 and 32 decapod species were recorded from each  
71 of six shallow-water cobble sites in Norway, England, Ireland and Italy (Robinson and Tully  
72 2000b; Linnane et al. 2001; Ringvold et al. 2015), while the faunal descriptions of another  
73 three sites in England, France and Ireland included a total of 382, 140 and 132 taxa  
74 respectively (George et al. 1995; Linnane et al. 2003; Foveau and Dauvin in press).

75

76 A similar situation is found in the Mediterranean Sea, where pebble bed habitats are also  
77 considered to be faunally impoverished (Bellan-Santini et al. 1994, 2002) despite the paucity  
78 of biological information on these assemblages (Bellan-Santini et al. 2002; Relini and  
79 Giaccone 2009). Given the presumption that cobble and pebble beds are depauperate, these  
80 habitats were not included in the reference list of Mediterranean marine habitats of  
81 conservation interest produced in connection with Barcelona Convention (UNEP-MAP-  
82 RAC/SPA 2006a). Yet in 2006, a population of the Maltese top-shell *Gibbula nivos*a was  
83 discovered from a pebble bed in Marsamxett, Malta (Evans et al. 2010, 2011). This is a  
84 critically endangered species endemic to Maltese waters, which has been afforded protection  
85 through the Bern Convention, the Barcelona Convention, and the European Union's 'Habitats  
86 Directive' (as an Annex II species) due to its risk of extinction (Evans et al. 2011).  
87 Subsequent work confirmed the importance of shallow-water cobbles and pebbles as a habitat  
88 for *G. nivos*a (Evans and Schembri 2014), indicating that pebble beds can be of conservation  
89 interest and highlighting the need for characterisation studies of these habitats.

90

91 Within this context, the present study was carried out to characterise the physical and  
92 biological features of sublittoral pebble beds in the Maltese Islands, thus providing detailed  
93 habitat data for Mediterranean sublittoral pebble beds for the first time, and enabling  
94 assessment of their species diversity. A second objective of this study was to identify  
95 correlations between the physical parameters and biotic components of the habitat.  
96 Recognition of such patterns is a necessary precursor for the formulation of hypotheses on the  
97 relationship between the physical nature of the pebble habitat and the species assemblage it  
98 supports (Underwood et al. 2000). Knowledge on the correlation between species  
99 assemblages and environmental characteristics is also vital for conservation efforts, since it

100 enables prediction of how these assemblages may change in response to environmental  
101 changes, including those associated with anthropogenic activities.

102

103

## 104 **Methods**

105

### 106 *Study sites*

107 A preliminary snorkelling survey was made along the low-lying coast of the Maltese Islands  
108 to map sublittoral pebble beds. Nineteen sites (Figure 1) were selected for further study,  
109 based on the following criteria: (i) the site consisted predominantly of pebbles (stones having  
110 a diameter of 2–10 cm following the UNEP-MAP-RAC/SPA [2006b] criteria), which lacked  
111 a covering of erect macrophytes; (ii) the site had an area of at least 25 m<sup>2</sup> of pebble habitat  
112 present at a depth of more than 0.5 m below chart datum. Areas with cobbles or small  
113 boulders that had a permanent cover of erect algae were excluded because they are  
114 considered to belong to a different assemblage – that of infralittoral algae (e.g. Bellan-Santini  
115 et al. 1994).

116

### 117 *Sediment sampling and laboratory analysis*

118 At each site, divers measured water depth using a digital depth gauge and estimated the  
119 dimensions of the pebble beds using fibreglass tape measures. Four replicate samples of the  
120 substratum were collected from each site; their position was selected at random, but no  
121 samples were collected within 2 m of the perimeter of the beds to avoid edge effects. A 0.1-  
122 m<sup>2</sup> circular sampler (modified from Borg et al. 2002) was pushed into the substratum to  
123 delineate the sampling area and prevent escape of motile invertebrates. Pebbles were then  
124 hand-collected and transferred to a 0.5-mm mesh bag, while a small hand net was used to

125 scoop the basal layer of finer granules into a separate 0.5-mm mesh bag. A suction sampler  
126 was simultaneously employed to collect highly-motile organisms, thus ensuring quantitative  
127 sampling of the whole assemblage. The thickness of the pebble layer was estimated by  
128 measuring the thickness of the exposed section in comparison with the adjacent undisturbed  
129 region. The 0.1-m<sup>2</sup> sampler allowed quantitative sampling of pebbles >8 mm, but not of finer  
130 sediments. Therefore an intact core sample (area: 0.0016 m<sup>2</sup>) of the granule layer and  
131 underlying fine sediments up to a depth of 5 cm was also collected for grain-size analysis. All  
132 fieldwork was carried out between July and September 2011.

133

134 Samples for biotic analysis were preserved in 10% formaldehyde in seawater. They were  
135 subsequently sorted for macrofauna (retained by a 0.5-mm sieve; e.g. Castelli et al. 2003),  
136 retaining all pebbles >8 mm for granulometric analysis. The biota were identified to the  
137 lowest taxon possible and enumerated to construct a taxon-abundance matrix and obtain  
138 estimates of number of taxa and abundance per sample.

139

140 For particle-size analysis, all pebbles >8 mm (collected using the 0.1-m<sup>2</sup> sampler) were  
141 manually sorted into size classes at half-phi intervals on the Udden-Wentworth scale using a  
142 gravel sizing template (Hydro Scientific Ltd.). Particles <8 mm (collected using the 0.0016-  
143 m<sup>2</sup> sampler) were treated with sodium hexametaphosphate, wet-sieved for the determination  
144 of silt content, then dried and separated into size classes at phi-intervals using a series of  
145 nested test-sieves (see Bale and Kenny 2005). Since the finer sediment was collected using  
146 smaller corers, the masses of the particle-size fractions <8 mm were scaled up using the ratio  
147 of the area of the two corers as the scaling factor. Grain-size data were analysed using the  
148 method of moments to obtain the geometric mean particle size, using the GRADISTAT  
149 program (Blott and Pye 2001). Since bimodal distributions were common, the mean particle

150 size for the gravel fraction (particles >2 mm) was calculated independently of any finer  
151 particles, which were then quantified separately in terms of the percentage sand and silt  
152 content.

153

154 Measurements of other sediment characteristics related to pebble shape and roughness were  
155 undertaken since these determine the way pebbles are ‘packed’ in a bed, and hence the shape  
156 and size of interstitial spaces (and therefore microhabitats) available. These measurements  
157 were made on subsamples consisting of ten pebbles randomly selected from each size  
158 fraction (>8 mm), for a total of 50 pebbles per subsample. Surface area was estimated from  
159 the maximum dimensions ( $L$ ,  $W$ ,  $H$ ) of the particles according to the equation:  $Surface\ Area =$   
160  $1.15*(LW+LH+WH)$  (see Graham et al. 1988). Stone resistance to rotation was calculated as  
161 the moment of inertia of an ellipsoid ( $I = m*(b^2+c^2)/5$ ; where  $m$  is the mass, and  $b$  and  $c$  are  
162 the lengths of the two minor semi-axes). Particle shape was characterised by: (i) form, in  
163 terms of elongation ( $W/L$ ) and flatness ( $H/W$ ) (Blott and Pye 2008); (ii) roundness, using a  
164 visual comparator (Blott and Pye 2008); and (iii) sphericity, as the square-root of the ratio of  
165 the largest inscribed circle diameter to the smallest circumscribed circle diameter (Riley  
166 1941), averaged across the three dimensions of the pebbles. Percentage cover of encrusting  
167 and filamentous algae was also recorded.

168

#### 169 *Physico-chemical characterisation of the water*

170 Sampling was undertaken at monthly intervals over a period of one year (July 2011 – June  
171 2012). Temperature, salinity, dissolved oxygen and turbidity were measured using an *in situ*  
172 meter (YSI 6820 multiparameter sonde) deployed just above the seafloor and two replicate  
173 seawater samples were collected from just above the pebble habitat using a Van Dorn water  
174 sampler. Nitrate and phosphate levels were determined using an automated continuous flow

175 analyser (Skalar SAN++ coupled with an SA1000 autosampler). Annual average  
176 concentrations for these parameters were calculated following standard practises in water  
177 quality monitoring (E.C. 2009). While physico-chemical parameters are not properties of the  
178 pebble beds *per se*, they were also taken into consideration since some of the study sites were  
179 within a harbour area (Sites 6–11; see Figure 1) and likely receive higher nutrient inputs and  
180 therefore have higher productivity, while Site 5 was situated close to a thermal effluent which  
181 could also affect its biota.

182

### 183 *Statistical analyses*

184 Univariate indices of diversity were calculated for each site, and for the pebble-bed  
185 assemblage as a whole (across all sites). These included: observed taxon richness ( $S_{\text{obs}}$ ),  
186 estimated total richness ( $S_{\text{est}}$ ), total abundance ( $TA$ ), diversity (Hill diversity  ${}^1D$ ), evenness  
187 ( ${}^1D/S_{\text{obs}}$ ) and relative evenness (Pielou  $J'$ ). The *Chao1* estimator (Chao 1984) was used to  
188 estimate  $S_{\text{est}}$  (see Foggo et al. 2003), while choice of evenness measures was based on the  
189 rationale of Jost (2010). Since functionally redundant species tend to be taxonomically  
190 related (Clarke and Warwick 1998), indices that take species' relatedness into account  
191 provide a broader view of biodiversity that potentially also includes functional aspects  
192 (Somerfield et al. 2008). Thus, taxonomic diversity ( $\Delta$ ), taxonomic distinctness ( $\Delta^*$ )  
193 (Warwick and Clarke 1995) and average variation in taxonomic distinctness ( $\Lambda^+$ ) (Clarke and  
194 Warwick 2001) were also estimated.

195

196 Multivariate analyses were carried out using the PRIMER v6 software (Clarke and Gorley  
197 2006) with PERMANOVA+ v1 add-on (Anderson et al. 2008). For biotic data, analyses were  
198 based on a Bray-Curtis similarity resemblance matrix calculated on square-root transformed  
199 taxon-abundance data. The taxa that were characteristic of the pebble-bed assemblage were

200 determined using SIMPER analysis (Clarke 1993), while nMDS ordination was performed to  
201 visualise the community pattern. The BVStep routine was subsequently used to find the  
202 smallest possible subset of taxa that together describe most of the among-site variation  
203 observed in the full dataset, using the “peeling” procedure as described in Clarke and  
204 Warwick (1998). The ordination pattern of sites based on physical attributes was visualised  
205 through PCA. Correlations between the similarity matrices obtained using the physical data  
206 (based on Euclidean distance) and biotic data (based on Bray-Curtis) were tested using a non-  
207 parametric form of the Mantel test (RELATE).

208

209 Distance-based multiple regression and redundancy analyses (DistLM and db-RDA; McArdle  
210 and Anderson 2001) were then used to identify the physical variables correlated with  
211 variation in the biotic assemblage. The analyses were made using the entire faunal dataset,  
212 and then repeated for sub-sets consisting of mobile and non-mobile fauna analysed  
213 separately. Non-mobile fauna included taxa that are either sedentary or permanently attached;  
214 parasitic taxa were omitted from these analyses. Although the DistLM/db-RDA routines do  
215 not require the assumption of multivariate normality, predictor variables should not be  
216 heavily-skewed or contain extreme outliers (Anderson et al. 2008). Therefore, prior to these  
217 analyses, Shapiro-Wilk normality tests were run and, where necessary, Box-Cox normalising  
218 transformations were applied (see Sokal and Rohlf 1995). Since abiotic variables were  
219 measured on different measurement scales they were also standardised to unit variance to  
220 bring them to a common scale (Legendre and Legendre 1998). DistLM was carried out using  
221 the transformed environmental variables after checking for multi-collinearity (no variables  
222 were removed since Pearson’s  $r$  was  $<0.9$  in all cases). Forward sequential fitting was used to  
223 select those variables with the highest explanatory power according to the adjusted- $R^2$   
224 selection criterion. A db-RDA plot was subsequently produced to allow visualization of the

225 sites' ordination according to the multivariate regression models previously generated via  
226 DistLM. An unconstrained ordination plot was produced via non-metric multidimensional  
227 scaling (nMDS) for comparison with the constrained db-RDA plot.

228

229

## 230 **Results**

231

### 232 *Physical characterisation*

233 A summary of the various physical parameters quantified for the pebble-bed sites is provided  
234 in Table 1. Seven sites occurred at a water depth <2.5 m, while the rest were found at a water  
235 depth of 5–12 m. Most of the pebble beds covered a relatively small area (25–500 m<sup>2</sup>)  
236 although two extensive beds (>3000 m<sup>2</sup>) were present in Marsamxett Harbour. In all cases,  
237 the pebble bed had a layered structure, with an upper stratum of pebbles and a lower stratum  
238 of granules and sand that became progressively finer with sediment depth; a basal silty layer  
239 was also present in the deeper sites (Figure 2). The upper pebbly stratum consisted of around  
240 two to three layers of pebbles with an overall thickness of ca. 5–10 cm. These pebbles mostly  
241 fell within the 16–32 mm or 32–64 mm size classes, although some variation in mean pebble  
242 size was evident between sites. The six sites located within Marsamxett Harbour had the  
243 highest proportion of sand and silt and the highest algal cover, while shallower sites had the  
244 lowest content of fine sediment and the lowest algal cover. Shallow sites also had the highest  
245 total surface area, reflecting the slightly thicker pebble beds present at these sites.

246

247 In terms of particle-shape characteristics, the pebbles from all sites classified as “slightly  
248 elongate”, “slightly flat”, “rounded” and “moderately spherical”, according to Blott and Pye's  
249 (2008) classification systems. There was minimal variation in annual mean values for

250 salinity, dissolved oxygen, turbidity and phosphate content between sites. Similar  
251 temperature values were also recorded at the different sites except at site 5, where the  
252 presence of a power station thermal effluent close to the pebble bed resulted in temperature  
253 being around 3°C higher. Some variation in levels of nitrate were also noted, with shallow  
254 sites having the highest values overall, although all values were within the expected range for  
255 Maltese inshore waters (Axiak 2004).

256

### 257 *Biotic characterisation*

258 A total of 39,993 individuals belonging to 332 macrofaunal taxa were recorded. Of these,  
259 34,035 individuals (313 taxa) were recorded from the upper pebble stratum while 5,958  
260 individuals (187 taxa) were found in the underlying layer of granules and finer sediments.  
261 The most diverse groups were the Mollusca (134 taxa), Crustacea (93 taxa) and Polychaeta  
262 (77 taxa). Spirorbinae spp. accounted for 45% of all individuals and 88% of the polychaetes,  
263 with nearly half of the spirorbids being recorded from a single site (Marsaxlokk; Site 4 in  
264 Figure 1). Polychaeta was the most abundant faunal group overall, but non-spirorbid  
265 polychaetes comprised only 6% of the total fauna (Figure 3). Other faunal groups included  
266 the Echinodermata, Cnidaria, Sipuncula, Nemertea, Tunicata and Actinopterygii which  
267 together accounted for only 1.5% of the total abundance. A larger richness of mobile fauna  
268 (246 taxa) was recorded compared to non-mobile fauna (82 taxa), but the total abundances of  
269 the two functional groups were very similar (49% mobile; 51% non-mobile).

270

271 The gradient of a taxon accumulation plot for the sampled sites (Figure 4) decreased after the  
272 fifth site, but did not reach an asymptote, with *Chao1* estimating a total taxon richness of 440  
273 taxa across all 19 sites. The actual number of taxa recorded per site ranged from 37 to 125,  
274 with an overall mean of 81 taxa. Sites located at depths <2.5 m had approximately half the

275 number of taxa (mean: 49 taxa) as those found in waters >5 m deep (mean: 96 taxa), but a  
276 higher abundance was recorded from the shallower sites. This was mainly due to high  
277 abundance values of one or two dominating taxa at the shallower sites. Evenness values were  
278 overall low ( $\max {}^1D/S_{\text{obs}} = 0.4$ ). Values of taxonomic diversity and distinctness were  
279 generally similar for all sites, with the exception of taxonomic diversity recorded from site 4  
280 which was much lower ( $\Delta = 6.8$ ) than that recorded elsewhere ( $\Delta >40$ ). The mean, minimum  
281 and maximum values of these univariate diversity indices recorded from the 19 sites are  
282 indicated in Table 2.

283

284 The taxa identified as main contributors to similarity between the 19 sites are shown in Table  
285 3; these are considered to be characteristic of the studied pebble-bed assemblages and include  
286 representatives of all three major taxonomic groups (Crustacea, Mollusca, Polychaeta) and  
287 one Echinodermata. These taxa were all recorded from at least 13 of the 19 sites, but only  
288 *Chondrochelia savignyi* and *Xantho pilipes* were found at all sites. The smallest subset of  
289 taxa that still captured the sample relationships seen in the full community pattern (BVStep  
290 using a cut-off of Spearman's  $\rho > 0.95$ ) consisted of the following 16 taxa: *Alvania*  
291 *mamillata*, *Amphipholis squamata*, *Ampithoe ramondi*, *Athanas nitescens*, *Bittium latreillii*,  
292 *Bittium reticulatum*, *Calcinus tubularis*, *Cerithium renovatum*, *Columbella rustica*, *Gibbula*  
293 *varia*, *Ischnochiton rissoi*, *Lysianassa costae*, *Maera grossimana*, *Melita hergensis*,  
294 *Microdeutopus* spp. and *Spirorbinae* spp. Several of these were in common with the list of  
295 taxa contributing to similarity between sites identified through SIMPER analysis. Excluding  
296 these 16 taxa and repeating the BVStep analysis did not yield another subset of taxa that  
297 could attain the Spearman's  $\rho > 0.95$  criterion, but resulted in a subset of 35 taxa that still  
298 closely matched the overall multivariate pattern (Spearman's  $\rho = 0.94$ ). The next iteration  
299 yielded a subset of 39 taxa with Spearman's  $\rho = 0.90$ ; subsequent iterations failed to identify

300 another subset that could adequately describe the overall multivariate pattern (Spearman's  $\rho <$   
301 0.80).

302

### 303 *Linking physical and biological patterns*

304 The resemblance matrices produced based on environmental parameters and taxon-  
305 abundance data were significantly correlated (RELATE: Spearman's  $\rho = 0.47$ ;  $p = 0.002$ )  
306 indicating that the measured physical variables may be important factors structuring the biotic  
307 assemblage. When considering predictor variables individually, the multivariate multiple  
308 regression made using the entire macrofaunal dataset indicated that the sand and silt content  
309 was the physical parameter that accounted for the highest variance in species data, followed  
310 by coverage of the pebble beds, percentage cover of algae on the pebbles, and depth of the  
311 beds (Table 4). When the environmental factors were added sequentially to build a  
312 parsimonious model correlated to the variation in biotic composition, the first chosen factor  
313 was the percentage sand and silt content. This was followed by patch area, depth and layer  
314 thickness, with the four factors explaining 50.6% of the variation in assemblage structure  
315 (Table 5). Almost identical results were obtained when mobile and non-mobile taxa were  
316 analysed separately, with the main difference being the inclusion of nitrate concentration in  
317 the parsimonious model for non-mobile taxa (Tables 4 and 5). The nMDS (unconstrained)  
318 and db-RDA (constrained) ordination plots of the sites based on the full biotic dataset are  
319 shown in Figure 5. Only the physical factors selected in the DistLM models were included in  
320 the db-RDA plot; the first two axes account for 80% of the fitted variation, and 40% of the  
321 total variation in assemblage structure.

322

323

## 324 **Discussion**

325

326 A total sampling area of 7.6 m<sup>2</sup> of pebble habitat yielded 332 different macrofaunal taxa and  
327 just under 40,000 individuals. These numbers contradict previous assertions that sublittoral  
328 pebble beds are species poor and depauperate habitats (e.g. Bellan-Santini et al. 1994). The  
329 percentage abundance values of faunal groups recorded from the Maltese pebble beds was  
330 comparable to those reported by Linnane et al. (2001) from cobble beds found at similar  
331 depths (7–17 m) in Norway, England and Italy, where crustaceans were the dominant motile  
332 fauna followed by molluscs and polychaetes; in contrast, molluscs were dominant at an Irish  
333 cobble site (Linnane et al. 2001, 2003). These authors provide estimates for the number of  
334 decapod species, ranging between 15–32 species (with sampling areas of 7–20 m<sup>2</sup>), similar to  
335 the 17 decapod species recorded by Robinson and Tully (2000a) from a different location in  
336 Ireland (depth 6–12 m; sampled area 5 m<sup>2</sup>), to the 23 species recorded by George et al. (1995)  
337 from Norfolk, England (depth 10–18 m; sampled area 7.3 m<sup>2</sup>), and to the 28 decapod species  
338 recorded from Maltese pebble beds (present work). Notwithstanding the wide geographical  
339 separation of these locations, a number of decapod taxa including *Athanas nitescens*, *Pisidia*  
340 spp. and *Xantho pilipes*, were common to several sites, and are therefore seemingly  
341 associated with pebble/cobble habitats.

342

343 The species richness recorded in the present study is comparable to that found in other  
344 Mediterranean habitats which are considered to be highly diverse, such as seagrass meadows  
345 and maerl beds. For instance, 244 macroinvertebrate species were recorded from a maerl bed  
346 in Maltese waters (sampling area = 4.8 m<sup>2</sup>; Sciberras et al. 2006), while a similar study on  
347 motile macroinvertebrates associated with *Posidonia oceanica* meadows recorded 315  
348 species (sampling area = 3.7 m<sup>2</sup>; Borg et al. 2010). Overall, it is clear that while pebble-bed  
349 habitats are generally held to be species poor, the few studies that have been made on these

350 habitats indicate that this is not the case; they are overlooked and under-studied habitats, but  
351 not necessarily impoverished ones. In addition, the number of species reported from cobble  
352 and pebble substrata is likely an underestimate of the total macrobenthic richness of these  
353 habitats, since none of the studies undertaken took habitat edges into account (George et al.,  
354 1995; Linnane et al. 2001, 2003; Robinson and Tully, 2000a; present study). Habitat edges  
355 may support a different suite of fauna, possibly having additional species beyond those  
356 occurring in the habitat centres. On the other hand, the edge species richness will also depend  
357 on the identity and richness of the adjacent habitats.

358

359 In the present study, species rich assemblages were recorded both at the level of individual  
360 sites and, when considering the entire habitat, across all sites. The high within-site diversity  
361 may be due to an intermediate disturbance regime (Osman 1977), or to the physically  
362 complex nature of the substratum (Robinson and Tully 2000a, 2000b) that incorporates  
363 interstitial spaces of various dimensions providing microhabitats and refugia from predators  
364 for animals of different sizes (Linnane et al. 2003; Liversage et al. 2017). Physical  
365 disturbance via wave action is probably the major cause of pebble overturn during winter  
366 months, but may play a minor role during prolonged periods of calm weather that occur in  
367 summer, especially in sheltered sites. Field observations made during the present sampling  
368 indicated that bioturbation, especially by the striped red mullet *Mullus surmuletus*, may be an  
369 important cause of disturbance in the deeper sites. The lack of erect algae on the surface of  
370 the pebbles, even during the calmer summer months, suggests that the pebbles are unstable  
371 and do overturn regularly.

372

373 Physical disturbance may itself increase the habitat complexity by leading to stratification (as  
374 observed in the present study) through vertical winnowing, which involves loss of fine

375 material to the subsurface by passing down through the interstitial cavities between the  
376 pebbles as these are disturbed by water motion or bioturbation (Parker and Klingeman 1982).  
377 Stratification may also be partly due to the selective fine particle entrainment, since this  
378 renders the surface layer coarser than the subsurface material. Such mechanisms enable the  
379 pebbles to persist in an unburied state (Parker and Klingeman 1982). Stratification can have  
380 important biological consequences since benthic community structure is often related to  
381 sediment textural characteristics (Anderson 2008), which could lead to different species  
382 groups being present in the different strata at a given site, thus increasing species diversity.

383

384 Most of the characteristic species of Maltese pebble beds were included in the smallest subset  
385 of taxa that still adequately described the among-site variation observed in the full dataset.  
386 This suggests that differences between the 19 study sites are partly driven by changes in  
387 abundance of these main taxa. Nonetheless, an element of structural redundancy (*sensu*  
388 Clarke and Warwick 1998) was also present, given that two other species subsets that  
389 included a total of 74 taxa between them also closely matched the overall multivariate  
390 pattern. In addition, several taxa were rare, with over half of the taxa being recorded from  
391 only one to three sites. Due to their rarity, such taxa may not have contributed substantially to  
392 the overall multivariate pattern but they are important contributors to the overall diversity of  
393 the pebble-bed assemblages.

394

395 The environmental parameters and taxon-abundance data were significantly correlated,  
396 indicating that at least some of the measured physical variables may be important factors  
397 structuring the biotic assemblage. One of the main factors which was highly correlated with  
398 biotic variation was the sand and silt content, probably because it is a good proxy for the  
399 hydrodynamic regime present at a site and may also influence food availability. Other

400 physical variables included in the parsimonious models were patch area, depth, pebble layer  
401 thickness. Depth can be a proxy for several other factors, including wave energy, light  
402 intensity and phytoplankton concentration, all of which may have an influence on benthic  
403 assemblages. The pebble layer thickness is a measure of habitat complexity. On its own, this  
404 factor explained less than 10% of the biotic variation but could explain almost 7% of the  
405 variation over and above that accounted for by the other parameters already in the model. The  
406 inclusion of coverage in the models is interesting given that the pebble patches are essentially  
407 habitat islands surrounded by other habitat types and, according to the classic theory of island  
408 biogeography, island area is one of the main factors influencing the occurrence and  
409 abundance of species in islands (MacArthur and Wilson 1967). This may be due to patch area  
410 *per se*, but can also result from its correlation with other factors that directly regulate the  
411 community structure (Ricklefs and Lovette 1999).

412

413 In conclusion, the present study shows that claims that sublittoral pebble beds are  
414 impoverished do not stand up to scrutiny, especially in the case of beds found deeper than a  
415 couple of metres. This misconception is likely due to the absence of detailed studies on these  
416 habitats. Indeed, a recent study on mobile gravels and pebbles in north-western France (25–66  
417 m depth) reached a similar conclusion that these habitats support a “surprisingly diversified  
418 macrofauna” (Foveau and Dauvin in press). The presence of unique or rare species within  
419 pebble beds would further enhance their conservation value, but this can only be ascertained  
420 through detailed comparative studies of the species assemblages found in different habitats,  
421 which was beyond the scope of the present work. On the other hand, pebbles beds may still  
422 represent unique assemblages with different functionality, even if none of their component  
423 species occur solely in pebble beds.

424

425 Our results indicate that sublittoral pebble beds can be highly diverse, with the biotic  
426 assemblage responding to a complex set of environmental variables. In such a situation,  
427 changes to the physical environment can lead to a significant impact on the assemblage  
428 structure and composition. In particular, the results of this study indicate that pebble-bed  
429 macrofaunal assemblages are sensitive to changes in hydrodynamic conditions and sediment  
430 loading, to alterations to the stratification of the pebble beds, and to fragmentation of the  
431 habitat patches. This information will enable better understanding of the ecological  
432 importance of pebble beds and of the potential impacts that may be caused by anthropogenic  
433 disturbance, thereby enabling more informed decisions for habitat conservation and  
434 management. In the case of the Maltese Islands, live populations of the critically endangered  
435 endemic top-shell *Gibbula nivos*a have only been recorded from cobble and pebble habitats  
436 since the rediscovery of this species in 2006 (Evans et al. 2010, 2011; Evans and Schembri  
437 2014; this study). Since *G. nivos*a is included in Annex II of the European Union's 'Habitats  
438 Directive' (species whose conservation requires designation of protected areas), infralittoral  
439 pebble beds should actually be considered as priority habitats when formulating conservation  
440 management plans and designating protected areas in Maltese coastal waters.

441

442

#### 443 **Compliance with ethical standards**

444

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448 **Ethical approval:** This article does not contain any studies with animals performed by any of  
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450

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454

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591

592 **Tables**

593

594

595 **Table 1**

596 Mean ( $\pm$  SD), minimum and maximum values of various physical parameters recorded from  
 597 sublittoral pebble beds at 19 sites in the Maltese Islands.

	<b>Mean <math>\pm</math> SD</b>	<b>Range</b>
Depth (m)	5.0 $\pm$ 3.4	0.7 – 12.0
Patch Area (m <sup>2</sup> )	806 $\pm$ 1422	25 – 3900
Thickness (cm)	5.28 $\pm$ 1.65	2.0 – 8.8
Mean Pebble Size (mm)	30.7 $\pm$ 15.0	12.8 – 68.0
Sand & Silt content (%)	18.9 $\pm$ 12.8	0.0 – 40.8
Mean Moment of Inertia (g cm <sup>2</sup> )	198 $\pm$ 419	6.8 – 1511
Total Surface Area (m <sup>2</sup> )	0.54 $\pm$ 0.20	0.3 – 1.0
Mean Elongation	0.74 $\pm$ 0.02	0.7 – 0.8
Mean Flatness	0.66 $\pm$ 0.04	0.6 – 0.7
Mean Roundness	0.69 $\pm$ 0.05	0.6 – 0.7
Mean Sphericity	0.75 $\pm$ 0.01	0.7 – 0.8
Filamentous algal cover (%)	6.5 $\pm$ 4.3	0.0 – 13.9
Encrusting algal cover (%)	14.0 $\pm$ 19.7	0.0 – 55.2
Annual Mean Temperature (°C)	20.65 $\pm$ 0.76	19.9 – 23.6
Annual Mean Salinity (psu)	37.94 $\pm$ 0.13	37.6 – 38.1
Annual Mean Dissolved Oxygen (%)	107.2 $\pm$ 5.1	93.4 – 113.7
Annual Mean Turbidity (NTU)	0.52 $\pm$ 0.58	0.1 – 2.7
Annual Mean [Nitrate] ( $\mu$ g L <sup>-1</sup> )	78.4 $\pm$ 115	15.7 – 513.5
Annual Mean [Phosphate] ( $\mu$ g L <sup>-1</sup> )	2.6 $\pm$ 1.3	1.7 – 7.0

598

599

600

601 **Table 2**602 Mean ( $\pm$  SD), minimum and maximum values for various univariate diversity indices

603 recorded from sublittoral pebble beds at the 19 study sites.

	<b>Mean <math>\pm</math> SD</b>	<b>Range</b>
Observed taxon richness - $S_{\text{obs}}$	81 $\pm$ 27	37 – 125
Estimated taxon richness - $S_{\text{est}}$	122 $\pm$ 37	59 – 185
Total abundance - $TA$ (ind. 0.4 m <sup>-2</sup> )	2105 $\pm$ 1996	409 – 9279
Hill diversity - ${}^1D$	13.7 $\pm$ 9.1	1.3 – 38.8
Evenness - ${}^1D/S_{\text{obs}}$	0.2 $\pm$ 0.1	0.0 – 0.4
Relative evenness - $J'$	0.5 $\pm$ 0.2	0.1 – 0.8
Taxonomic diversity - $\Delta$	65.2 $\pm$ 17.6	6.8 – 85.2
Taxonomic distinctness - $\Delta^*$	88.6 $\pm$ 4.7	79.7 – 98.4
Average variation in taxonomic distinctness - $\Lambda^+$	318.0 $\pm$ 40.7	261.6 – 421.0

604

605

606

607 **Table 3**608 Output from SIMPER analysis based on square-root transformed abundance data, identifying  
609 main taxa contributing to similarity in faunal assemblage present at the 19 study sites.

<b>Taxonomic group</b>	<b>Species</b>	<b>Mean Abundance (<math>\sqrt{\text{ind. } 0.4 \text{ m}^{-2}}</math>)</b>	<b>Frequency of Occurrence (%)</b>	<b>Mean Similarity</b>	<b>Contribution to Similarity (%)</b>
Polychaeta Sabellidae	Spirorbinae spp.	10.3	78.9	4.06	12.11
Crustacea Tanaidacea	<i>Chondrochelia savignyi</i>	5.5	100	3.11	9.27
Crustacea Amphipoda	<i>Gammarella fucicola</i>	3.0	94.7	1.39	4.14
Crustacea Decapoda	<i>Xantho pilipes</i>	1.7	100	1.04	3.11
Crustacea Amphipoda	<i>Ampithoe ramondi</i>	1.9	94.7	1.04	3.09
Mollusca Polyplacophora	<i>Ischnochiton rissoi</i>	2.1	84.2	0.99	2.95
Crustacea Amphipoda	<i>Microdeutopus spp.</i>	2.0	89.5	0.87	2.59
Crustacea Decapoda	<i>Athanas nitescens</i>	1.8	84.2	0.86	2.56
Crustacea Amphipoda	<i>Melita hergensis</i>	2.4	68.4	0.82	2.46
Polychaeta Nereididae	<i>Nereis rava</i>	1.4	84.2	0.74	2.19
Crustacea Amphipoda	<i>Maera grossimana</i>	2.0	68.4	0.73	2.18
Echinodermata Ophuroidea	<i>Amphipholis squamata</i>	1.7	84.2	0.72	2.16
Mollusca Gastropoda	<i>Gibbula varia</i>	1.7	68.4	0.70	2.10

610

611

612 **Table 4**

613 Results of DistLM analysis identifying physical variables correlated to the variation in biotic composition, based on the adjusted-R<sup>2</sup> criterion for  
 614 each variable taken individually (ignoring other variables), using (a) the entire macrofaunal dataset, (b) mobile taxa only, (c) non-mobile taxa  
 615 only. Analyses were performed using Bray-Curtis similarity to measure variance in biotic data. % var: percentage of variance in species data  
 616 explained by that variable; p-values lower than 0.05 are indicated in bold.

617

Variable	(a) All Fauna			(b) Mobile Fauna			(c) Non-mobile Fauna		
	% var	Pseudo-F	P	% var	Pseudo-F	P	% var	Pseudo-F	P
Depth	16.3	3.302	<b>0.004</b>	16.8	3.435	<b>0.003</b>	12.5	2.429	<b>0.027</b>
Layer Thickness	9.0	1.674	0.071	9.0	1.689	0.055	8.4	1.550	0.117
Patch Area	23.3	5.167	<b>0.001</b>	22.0	4.806	<b>0.001</b>	24.3	5.467	<b>0.001</b>
Mean Pebble Size	7.4	1.360	0.160	8.1	1.501	0.105	4.7	0.840	0.537
Sand & Silt content	24.3	5.447	<b>0.001</b>	22.4	4.912	<b>0.001</b>	24.6	5.536	<b>0.001</b>
Mean Elongation	2.9	0.504	0.983	3.4	0.596	0.910	2.7	0.480	0.929
Mean Flatness	5.3	0.951	0.463	5.5	0.990	0.445	4.4	0.777	0.623
Mean Roundness	6.5	1.181	0.257	5.5	0.992	0.420	7.9	1.453	0.151
Mean Sphericity	6.2	1.125	0.276	7.1	1.303	0.189	4.6	0.814	0.587
Mean Moment of Inertia	10.6	2.017	<b>0.031</b>	10.9	2.083	<b>0.023</b>	8.7	1.615	0.122
Total Surface Area	11.1	2.131	<b>0.031</b>	11.7	2.252	<b>0.012</b>	8.9	1.662	0.096
Filamentous Algal cover	16.7	3.404	<b>0.001</b>	16.4	3.325	<b>0.001</b>	15.6	3.150	<b>0.006</b>
Encrusting Algal cover	19.0	3.989	<b>0.001</b>	17.7	3.650	<b>0.002</b>	21.5	4.661	<b>0.001</b>
Annual Mean Temperature	6.6	1.207	0.216	8.3	1.547	0.065	4.0	0.708	0.692
Annual Mean Salinity	4.5	0.801	0.656	5.0	0.904	0.547	3.3	0.574	0.857
Annual Mean Dissolved Oxygen	6.5	1.173	0.248	6.2	1.128	0.302	5.5	0.991	0.409
Annual Mean Turbidity	6.5	1.185	0.237	6.6	1.197	0.250	6.8	1.248	0.237
Annual Mean Nitrate concentration	12.4	2.402	<b>0.010</b>	10.0	1.887	<b>0.041</b>	15.9	3.211	<b>0.009</b>
Annual Mean Phosphate concentration	14.9	2.971	<b>0.004</b>	14.7	2.932	<b>0.002</b>	15.0	3.008	<b>0.006</b>

618 **Table 5**

619 Results of DistLM analysis identifying physical variables correlated to the variation in biotic  
 620 composition, based on the adjusted-R<sup>2</sup> criterion with forward-selection of variables, where  
 621 the amount explained by each variable added to the model is conditional on variables already  
 622 in the model (i.e. those variables listed above it), using (a) the entire macrofaunal dataset, (b)  
 623 mobile taxa only, (c) non-mobile taxa only. Only variables up to the first highly non-  
 624 significant *p*-value (>0.09) are included in sequential fitting. Analyses were performed using  
 625 Bray-Curtis similarity to measure variance in biotic data. % var: percentage of variance in  
 626 species data explained by that variable; cum. %: cumulative percentage of variance  
 627 explained; *p*-values lower than 0.05 are indicated in bold.

628

Variable	(a) All Fauna			
	% var	cum %	Pseudo-F	P
Sand & Silt content	24.3	24.3	5.447	<b>0.001</b>
Patch Area	9.6	33.8	2.312	<b>0.001</b>
Depth	7.2	41.0	1.826	<b>0.013</b>
Layer Thickness	6.3	47.2	1.665	<b>0.039</b>
Mean Pebble Size	5.3	52.6	1.452	0.093
Variable	(b) Mobile Fauna			
	% var	cum %	Pseudo-F	P
Sand & Silt content	22.4	22.4	4.912	<b>0.001</b>
Patch Area	9.6	32.0	2.262	<b>0.002</b>
Depth	8.3	40.3	2.077	<b>0.007</b>
Mean Pebble Size	6.0	46.3	1.565	0.054
Layer Thickness	5.3	51.6	1.421	0.128
Variable	(c) Non-mobile Fauna			
	% var	cum %	Pseudo-F	P
Sand & Silt content	24.6	24.6	5.536	<b>0.001</b>
Layer Thickness	11.1	35.7	2.775	<b>0.003</b>
Patch Area	9.3	45.0	2.524	<b>0.005</b>
Annual Mean Nitrate concentration	6.7	51.7	1.935	<b>0.043</b>
Annual Mean Phosphate concentration	4.4	56.1	1.302	0.208

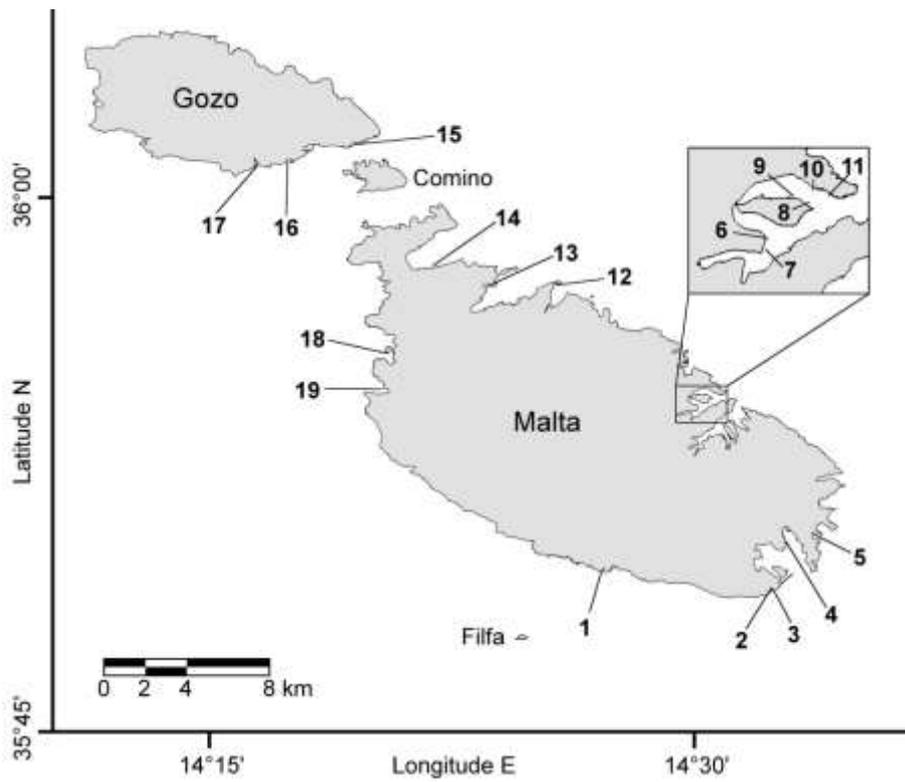
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632 **Figures with legends**

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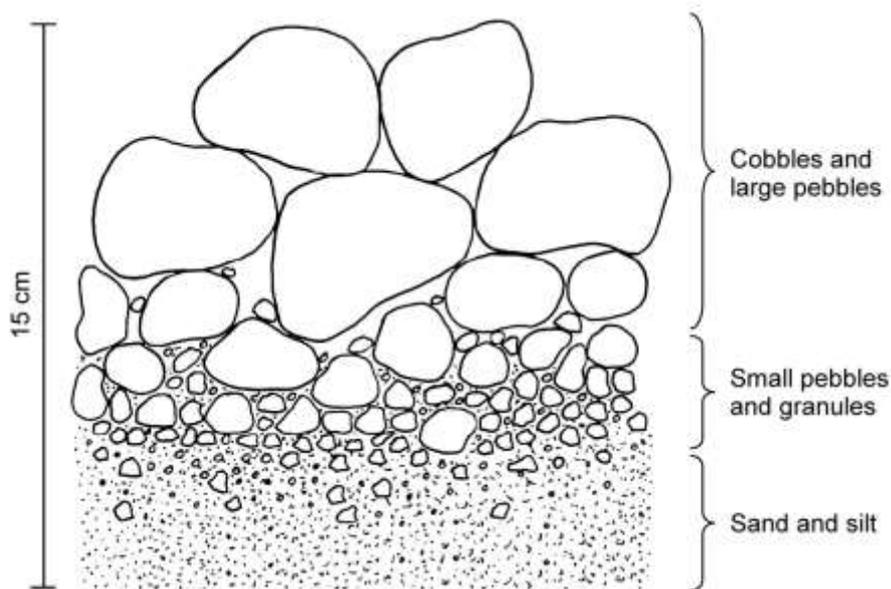
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636 **Figure 1** Map of the Maltese Islands indicating the location of the 19 sampling sites  
637 used in the present study. Inset shows enlarged view of Marsamxett Harbour.

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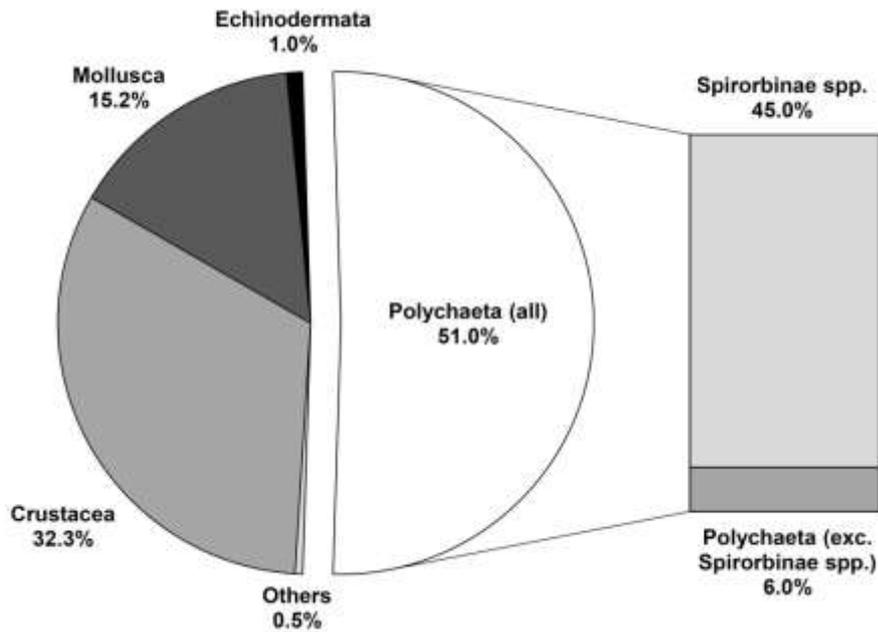
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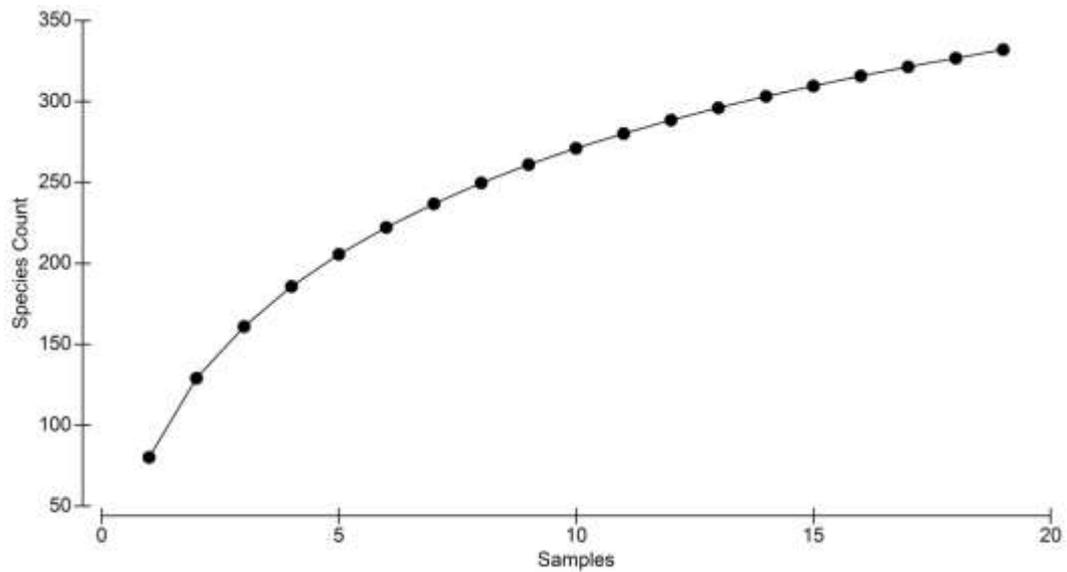
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642 **Figure 2** Diagrammatic pebble-bed profile showing vertical stratification, with an upper  
643 layer of pebbles and a lower layer of granules and sand; a basal layer of silty sand was  
644 typically present in harbour environments.



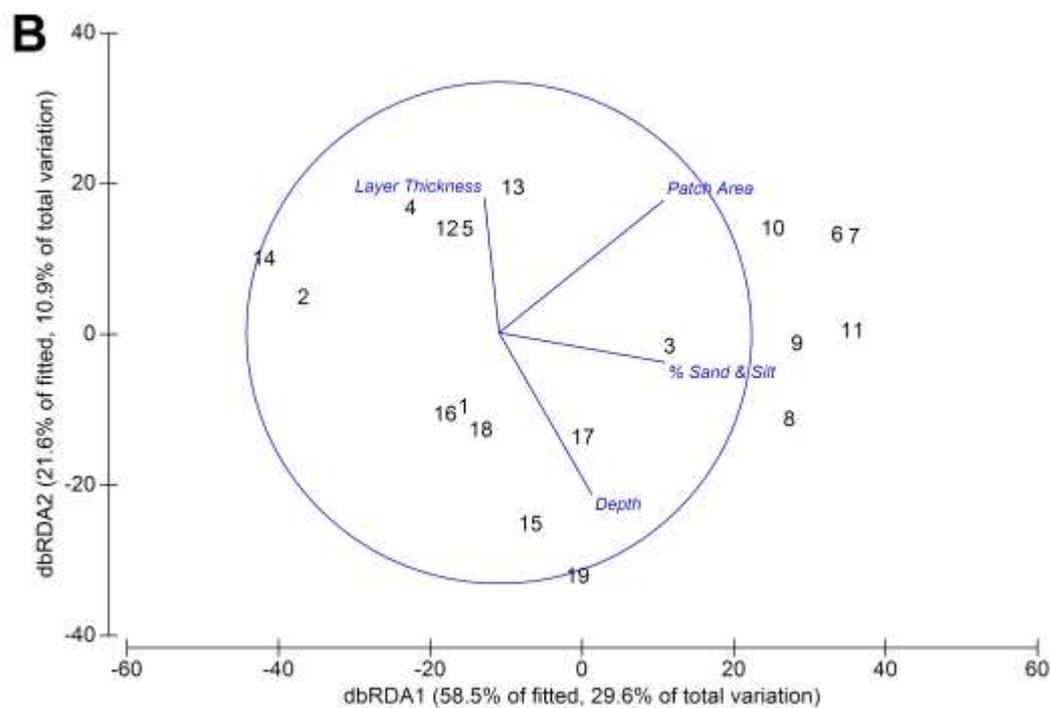
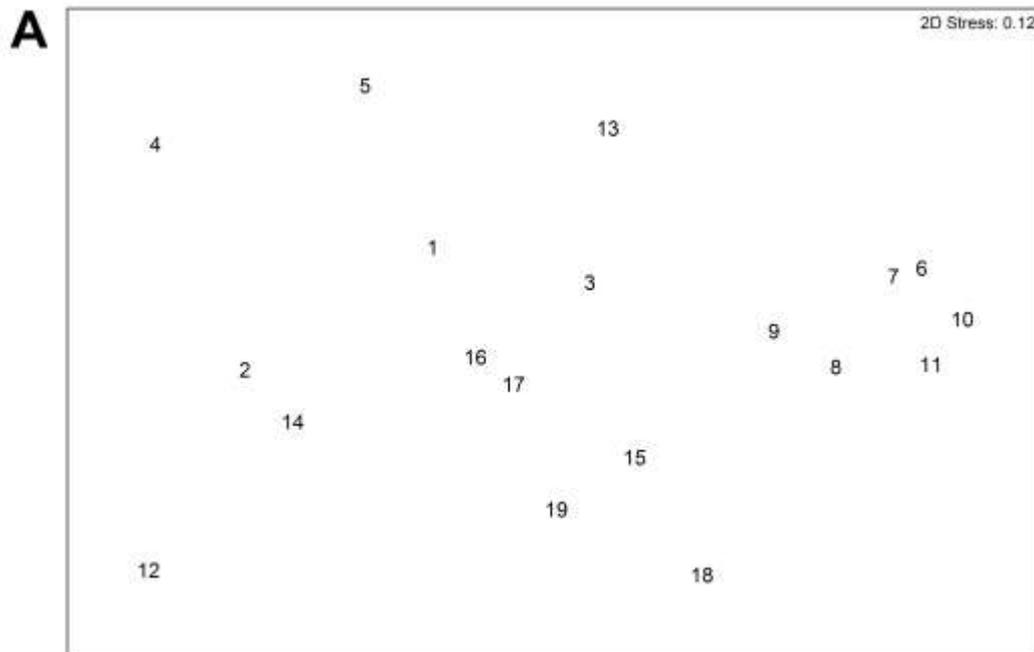
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**Figure 3** Percentage abundance of major faunal groups recorded from sublittoral pebble beds at the 19 study sites.



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**Figure 4** Taxon-accumulation plot showing cumulative number of taxa recorded from infralittoral pebble beds at the 19 study sites; the *Chao1* richness estimator indicated that the plot should reach an asymptote at a taxon richness of 440.



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**Figure 5** (a) Unconstrained non-metric multidimensional scaling ordination plot, where sites are positioned according to their similarity in biotic assemblage only. (b) Distance-based RDA ordination, where the ordination based on biotic data is constrained by their correlation with environmental variables, with vector projections of the physical factors selected by the DistLM routine; the length and direction of the vectors represent the strength and direction of the relationship between the variation in biotic assemblage and the physical variables. Both ordinations were performed using Bray-Curtis similarity of square-root transformed species counts.