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

and silt, with the area of the pebble bed, with water depth, and with the thickness of the

pebble layer. This indicates that pebble-bed macrofaunal assemblages are sensitive to

changes in hydrodynamic conditions and sediment loading, to alterations to the stratification

of the pebble beds, and to fragmentation of the habitat patches. These results contradict

assertions that sublittoral pebble beds are impoverished, instead showing that they can be

highly diverse habitats supporting biotic assemblages that respond to a complex set of

environmental variables. The present findings enable better understanding of the ecological

importance of pebble beds and of the potential impacts that may be caused by anthropogenic

disturbance, thereby enabling more informed decisions for habitat conservation and

management.

Keywords: Biodiversity, Conservation, Cobble bed, Community composition, Habitat,

Malta, Mediterranean

Introduction

The 1992 Rio de Janeiro Convention on Biological Diversity heralded the "era of

biodiversity" (Boero 2010) where exploration of diversity patterns and their environmental

correlates became more imperative than ever. Nonetheless, to date the spatial patterns of

marine benthic diversity remain poorly known for many habitats (Costello et al. 2010).

Sublittoral cobble and pebble beds are a case in point. Quantitative descriptions of cobble or

pebble biota are rare, possibly because conventional sampling methods such as cores or grabs

are not suitable for these habitats (Linnane et al. 2003). We are aware of only five studies

published in the mainstream literature that looked at the entire macrofaunal assemblage of

these habitats, based on sites in Canada, England, Ireland, France (Atlantic coast) and

Norway (Scheibling and Raymond 1990; George et al. 1995; Linnane et al. 2003; Ringvold et

al. 2015; Foveau and Dauvin in press). The few other studies that have been undertaken on

these habitats focused exclusively on specific groups such as macrophytes (Lieberman et al.

1979, 1984; Davis and Wilce 1987a, 1987b; Scheibling et al. 2009a, 2009b), sessile biota

(Osman 1977; Balazy & Kuklinski 2003, 2007), or decapods (Robinson and Tully 2000a,

2000b; Linnane et al. 2001). Of these, only Robinson and Tully (2000a) investigated the

spatial variability in community structure in relation to substratum characteristics, but they

also included areas that consisted predominantly of coarse sand or bedrock amongst their five

sites.

Consequently not much is known about the physical or biological characteristics of sublittoral

pebble and cobble habitats. These habitats are generally considered to be faunally

impoverished (Connor et al. 2004; Foveau and Dauvin in press), a view possibly extrapolated

from intertidal cobble and pebble habitats which have been described as "intertidal deserts"

where macrobiota are absent (Little 2000). Accordingly, habitat classification systems used

for marine conservation and management assume that there is little of interest in ecosystems

comprising pebbles and cobbles. Yet this view does not appear to have been borne out by

empirical studies, since the limited information available indicates that these habitats may not

be so depauperate. For instance, between 15 and 32 decapod species were recorded from each

of six shallow-water cobble sites in Norway, England, Ireland and Italy (Robinson and Tully

2000b; Linnane et al. 2001; Ringvold et al. 2015), while the faunal descriptions of another

three sites in England, France and Ireland included a total of 382, 140 and 132 taxa

respectively (George et al. 1995; Linnane et al. 2003; Foveau and Dauvin in press).

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 nivosa* 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. nivosa* (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^2 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^2 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^2 sampler allowed quantitative sampling of pebbles >8 mm, but not of finer

130 sediments. Therefore an intact core sample (area: 0.0016 m^2) 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^2 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^2 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_{obs}),

186 estimated total richness (S_{est}), total abundance (*TA*), diversity (Hill diversity ^1D), evenness

187 ($^1D/S_{obs}$) and relative evenness (Pielou *J'*). The *Chao1* estimator (Chao 1984) was used to

188 estimate S_{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 (Δ), taxonomic distinctness (Δ^*)

193 (Warwick and Clarke 1995) and average variation in taxonomic distinctness (Λ^+) (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

determined using SIMPER analysis (Clarke 1993), while nMDS ordination was performed to

visualise the community pattern. The BVStep routine was subsequently used to find the

smallest possible subset of taxa that together describe most of the among-site variation

observed in the full dataset, using the "peeling" procedure as described in Clarke and

Warwick (1998). The ordination pattern of sites based on physical attributes was visualised

through PCA. Correlations between the similarity matrices obtained using the physical data

(based on Euclidean distance) and biotic data (based on Bray-Curtis) were tested using a non-

parametric form of the Mantel test (RELATE).

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. The analyses were made using the entire faunal dataset,

and then repeated for sub-sets consisting of mobile and non-mobile fauna analysed

separately. Non-mobile fauna included taxa that are either sedentary or permanently attached;

parasitic taxa were omitted from these analyses. 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). Therefore, prior to these

analyses, Shapiro-Wilk normality tests were run 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 (Legendre and Legendre 1998). 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. A db-RDA plot was subsequently produced to allow visualization of the

9

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^2)

236   although two extensive beds (>3000 m^2) 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 $^1D/S_{obs}$ = 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 (Δ = 6.8) than that recorded elsewhere (Δ >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 ρ > 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 ρ >0.95 criterion, but resulted in a subset of 35 taxa that still

298   closely matched the overall multivariate pattern (Spearman's ρ = 0.94). The next iteration

299   yielded a subset of 39 taxa with Spearman's ρ = 0.90; subsequent iterations failed to identify

300   another subset that could adequately describe the overall multivariate pattern (Spearman's ρ <

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

A total sampling area of 7.6 m^2 of pebble habitat yielded 332 different macrofaunal taxa and

just under 40,000 individuals. These numbers contradict previous assertions that sublittoral

pebble beds are species poor and depauperate habitats (e.g. Bellan-Santini et al. 1994). The

percentage abundance values of faunal groups recorded from the Maltese pebble beds was

comparable to those reported by Linnane et al. (2001) from cobble beds found at similar

depths (7–17 m) in Norway, England 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), to the 23 species recorded by George et al. (1995)

from Norfolk, England (depth 10–18 m; sampled area 7.3 m^2), and to the 28 decapod species

recorded from Maltese pebble beds (present work). Notwithstanding the wide geographical

separation of these locations, a number of decapod taxa including *Athanas nitescens*, *Pisidia*

spp. and *Xantho pilipes*, were common to several sites, and are therefore seemingly

associated with pebble/cobble habitats.

The species richness recorded in the present study is comparable to that found in other

Mediterranean habitats which are considered to be highly diverse, such as seagrass meadows

and maerl beds. For instance, 244 macroinvertebrate species were recorded from a maerl bed

in Maltese waters (sampling area = 4.8 m^2; Sciberras et al. 2006), while a similar study on

motile macroinvertebrates associated with *Posidonia oceanica* meadows recorded 315

species (sampling area = 3.7 m^2; Borg et al. 2010). Overall, it is clear that while pebble-bed

habitats are generally held to be species poor, the few studies that have been made on these

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

In the present study, species rich assemblages were recorded both at the level of individual sites and, when considering the entire habitat, across all sites. The high within-site diversity may be due to an intermediate disturbance regime (Osman 1977), or to the physically complex nature of the substratum (Robinson and Tully 2000a, 2000b) that incorporates interstitial spaces of various dimensions providing microhabitats and refugia from predators for animals of different sizes (Linnane et al. 2003; Liversage et al. 2017). 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 in sheltered sites. Field observations made during the present sampling indicated that bioturbation, especially by the striped red mullet *Mullus surmuletus*, may be an important cause of disturbance in the deeper sites. The lack of erect algae on the surface of the pebbles, even during the calmer summer months, suggests that the pebbles are unstable and do overturn regularly.

Physical disturbance may itself increase the habitat complexity by leading to stratification (as 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 nivosa* 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. nivosa* 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

447 **Conflict of interest:** The authors have no conflict of interest.

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

449 the authors.

450

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Tables

Table 1

Mean (± SD), minimum and maximum values of various physical parameters recorded from

sublittoral pebble beds at 19 sites in the Maltese Islands.

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

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Table 2

Mean (± SD), minimum and maximum values for various univariate diversity indices

recorded from sublittoral pebble beds at the 19 study sites.

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

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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.

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

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Table 4

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

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	**0.004**	16.8	3.435	**0.003**	12.5	2.429	**0.027**
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	**0.001**	22.0	4.806	**0.001**	24.3	5.467	**0.001**
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	**0.001**	22.4	4.912	**0.001**	24.6	5.536	**0.001**
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	**0.031**	10.9	2.083	**0.023**	8.7	1.615	0.122
Total Surface Area	11.1	2.131	**0.031**	11.7	2.252	**0.012**	8.9	1.662	0.096
Filamentous Algal cover	16.7	3.404	**0.001**	16.4	3.325	**0.001**	15.6	3.150	**0.006**
Encrusting Algal cover	19.0	3.989	**0.001**	17.7	3.650	**0.002**	21.5	4.661	**0.001**
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	**0.010**	10.0	1.887	**0.041**	15.9	3.211	**0.009**
Annual Mean Phosphate concentration	14.9	2.971	**0.004**	14.7	2.932	**0.002**	15.0	3.008	**0.006**

Table 5

Results of DistLM analysis identifying physical variables correlated to the variation in biotic composition, based on the adjusted-R^2 criterion with 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), using (a) the entire macrofaunal dataset, (b) mobile taxa only, (c) non-mobile taxa only. Only variables up to the first highly non-significant *p*-value (>0.09) are included in sequential fitting. Analyses were performed using Bray-Curtis similarity to measure variance in biotic data. % var: percentage of variance in species data explained by that variable; cum. %: cumulative percentage of variance explained; p-values lower than 0.05 are indicated in bold.

| Variable	(a) All Fauna			
	% var	cum %	Pseudo-F	P
Sand & Silt content	24.3	24.3	5.447	**0.001**
Patch Area	9.6	33.8	2.312	**0.001**
Depth	7.2	41.0	1.826	**0.013**
Layer Thickness	6.3	47.2	1.665	**0.039**
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	**0.001**
Patch Area	9.6	32.0	2.262	**0.002**
Depth	8.3	40.3	2.077	**0.007**
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	**0.001**
Layer Thickness	11.1	35.7	2.775	**0.003**
Patch Area	9.3	45.0	2.524	**0.005**
Annual Mean Nitrate concentration	6.7	51.7	1.935	**0.043**
Annual Mean Phosphate concentration	4.4	56.1	1.302	0.208

Figures with legends

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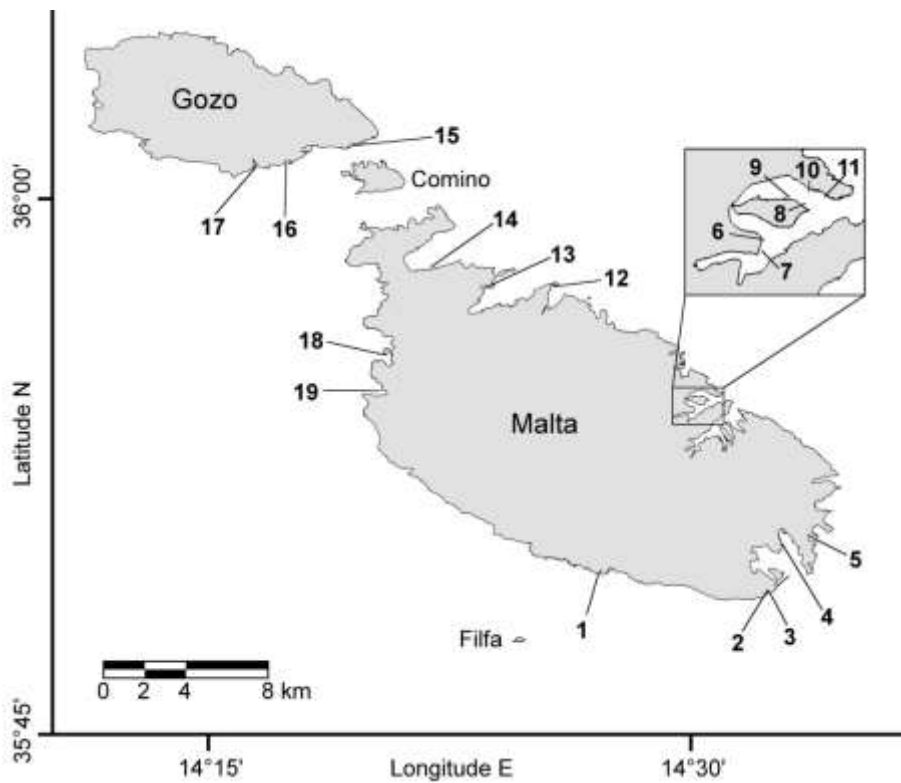
Figure 1   Map of the Maltese Islands indicating the location of the 19 sampling sites
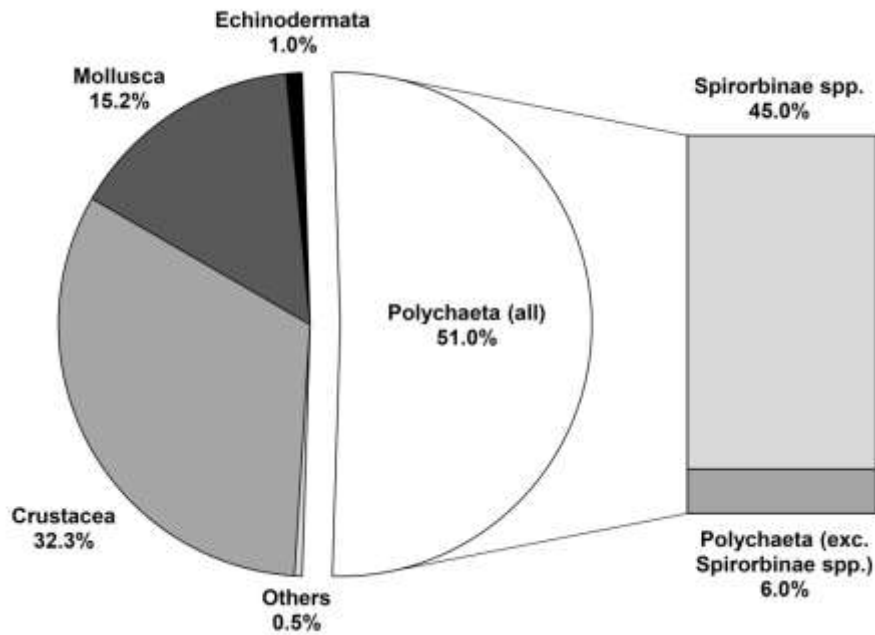used in the present study. Inset shows enlarged view of Marsamxett Harbour.

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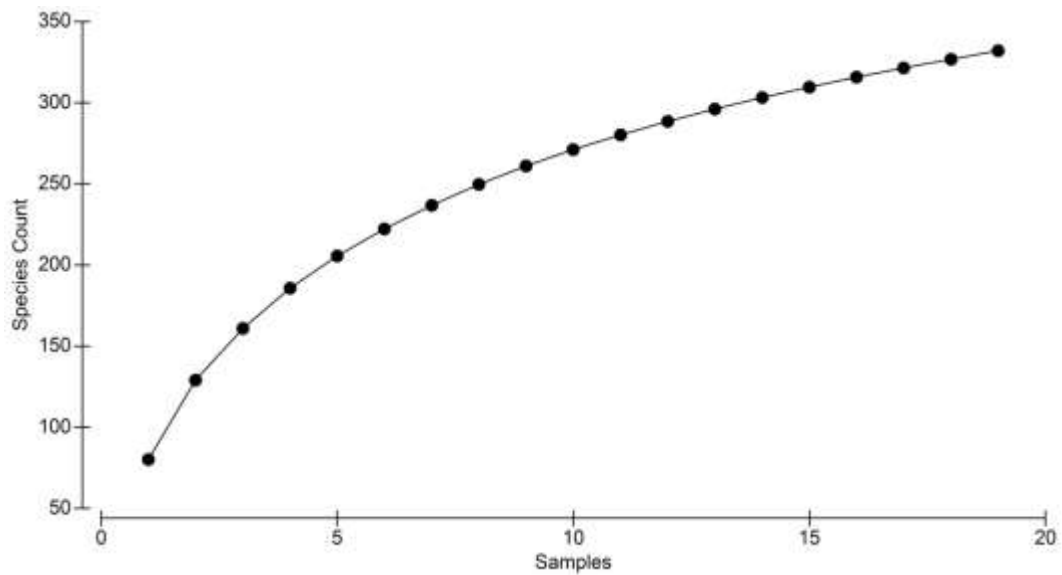641
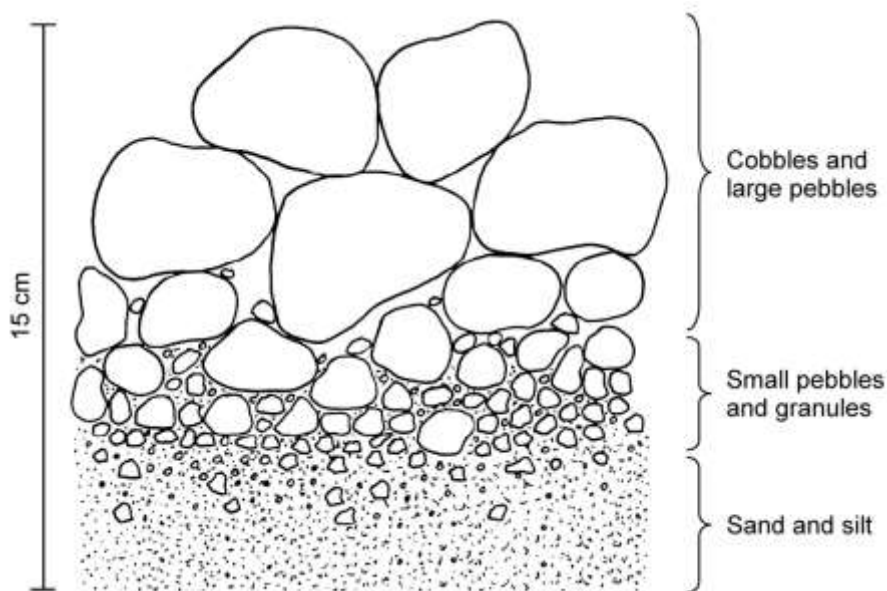
Figure 2   Diagrammatic pebble-bed profile showing vertical stratification, with an upper
layer of pebbles and a lower layer of granules and sand; a basal layer of silty sand was
typically present in harbour environments.

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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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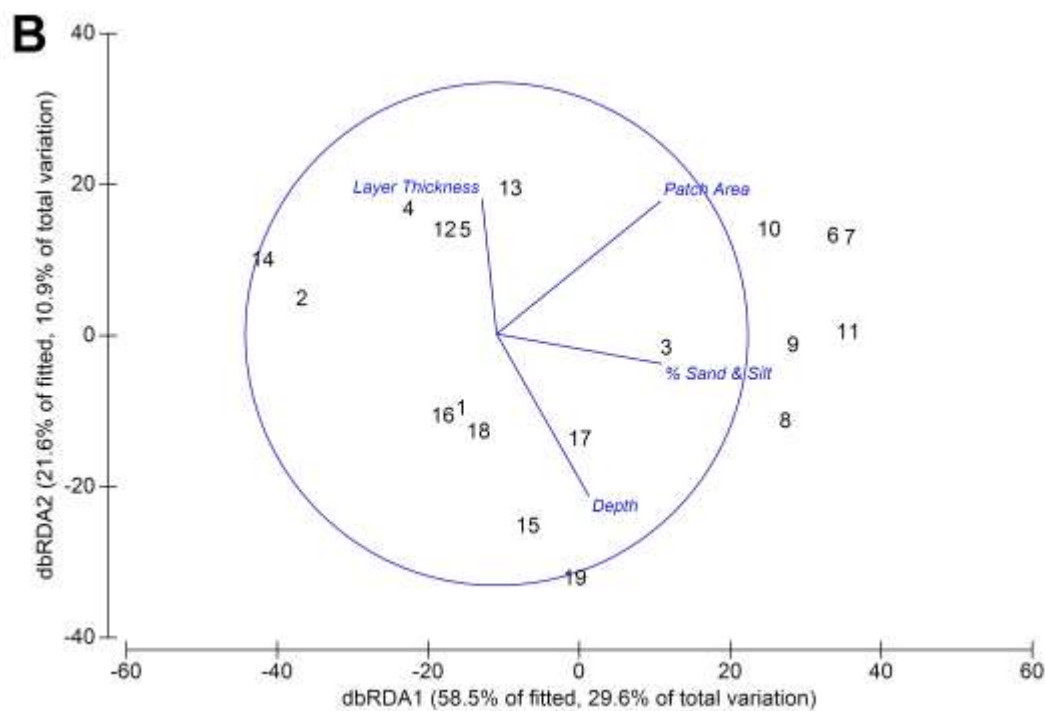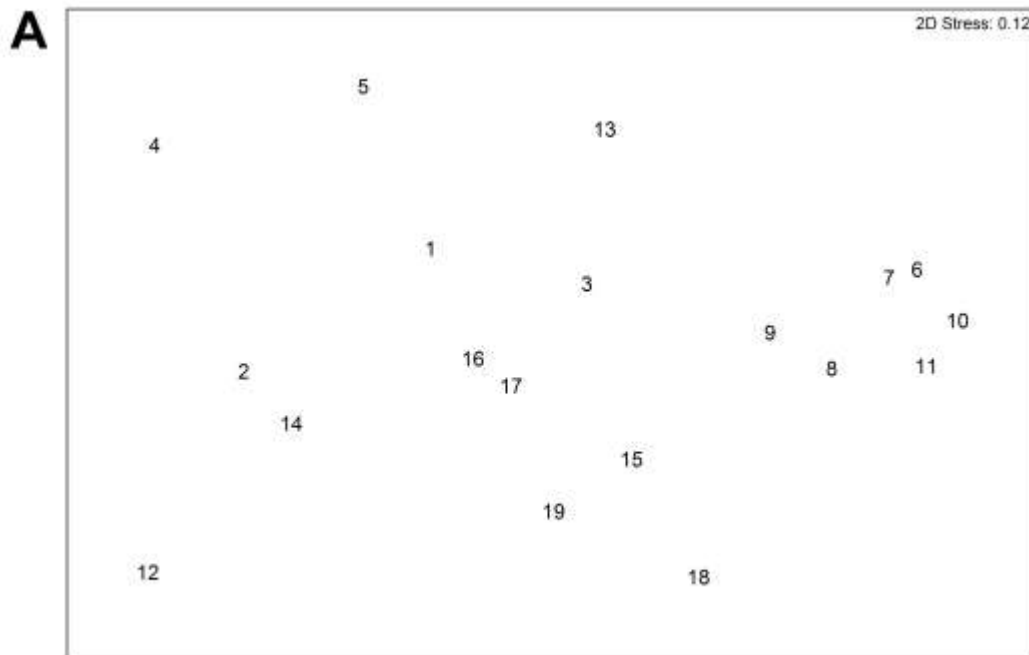
Figure 5 (a) Unconstrained non-metric multidimensional scaling ordination plot, where sites are positioned according to their similarity in biotic assemblage only. (b) Distance-based RDA ordination, where the ordination based on biotic data is constrained by their correlation with environmental variables, 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.