

1 **Assessing the conservation status of marine habitats: thoughts from a**
2 **sandflat on the Isles of Scilly**

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14 **Abstract**

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16 Statutory monitoring of the fauna of the ‘mudflats and sandflats not covered by seawater
17 at low tide’ biotope complex on St Martin’s Flats, a part of the Isles of Scilly Complex
18 Special Area of Conservation, was undertaken in 2000, 2004 and 2009. The targets set
19 by Natural England for “characteristic biotopes” were that “composite species, abundance
20 and diversity should not deviate significantly from an established baseline, subject to
21 natural change”. The three specified biotopes could not be distinguished, and instead
22 three assemblages were subjectively defined based on sediment surface features. There
23 were statistically significant natural changes in diversity and species composition
24 between years, especially in the association initially characterized by the razor-clam
25 *Ensis*, and possible reasons for this are discussed. It is suggested that setting fixed local
26 limits on natural variability is almost always impractical. Two possible approaches to
27 distinguishing between natural and anthropogenic changes are suggested; a change in
28 ecological condition as indicated by AMBI scores, and a significant change in average
29 taxonomic distinctness (Δ^+) compared with expectation. The determination of species
30 biomasses as well as abundances might also open more possibilities for assessment. The
31 practice of setting objectives for a marine SAC feature that include the range and number
32 of biotopes cannot be supported, in view the difficulty in ascribing assemblages to
33 recognised biotopes. A more realistic definition of species assemblages might best be
34 gained from examination of the species that *consistently* make a substantial contribution
35 to the Bray Curtis similarity among samples collected from specific sites.

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37 **Key words:** Marine benthos, biotope definition, diversity, species composition, natural
38 variability, AMBI scores, taxonomic distinctness

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42 1. Introduction

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44 The global Convention on Biological Diversity (CBD) held in Rio de Janeiro in 1992
45 (Secretariat of the Convention on Biological Diversity 2000) called for the creation and
46 enforcement of national strategies and action plans “to conserve, protect and enhance
47 biological diversity”. The European Union Directive 92/43/EEC (Habitats Directive) and
48 the Oslo and Paris Convention (OSPAR Commission 2008) have each encouraged
49 national commitments to marine biodiversity conservation. Although loss of biodiversity
50 is regarded as the main marine conservation issue (Ray & McCormick-Ray, 2014), the
51 practical implementation of such legislation has unfortunately sidestepped this issue, and
52 biodiversity *per se* has not been an explicit conservation attribute. The Habitats Directive
53 requires the maintenance or restoration of natural habitats and species of European
54 interest at “*favourable conservation status*”, with a network of Special Areas of
55 Conservation (SACs) being one of the main means of achieving this. Given that the
56 framework within which marine conservation is monitored and managed, and the
57 associated language, may not be familiar to scientists in the broader ecological
58 community it is worth giving a brief explanation.

59 Each SAC is designated because it is considered to contain examples of habitats listed in
60 Annexe 1 of the directive, or is essential for the maintenance of a population of a species
61 listed in Annexe 2. Within the UK these are collectively referred to as ‘*interest features*’
62 (Davies et al. 2001). Examples of features in the Isles of Scilly Complex SAC are
63 sandbanks and mudflats. For each feature at least one *conservation objective* is
64 formulated. This is a statement of what is to be achieved in terms of managing the
65 feature. Features may be broken down into *sub-features*. For each feature or sub-feature
66 certain *attributes* are defined, which are the measurable aspects of the feature which are
67 to be monitored. For each attribute certain *measures* are chosen which are considered to
68 be indicative of the overall health of the feature, and for each measure *target conditions*
69 are set. The purpose of monitoring in this framework, therefore, is to determine those
70 measures and see if they are consistent with the target conditions. If they are, the
71 conservation objectives are being met and the feature may be considered to be in
72 favourable status. A monitoring strategy for a feature must measure at least one attribute,
73 such as its extent, biotic composition, biological structure and physical structure (Davies
74 et al., 2001). Central to the delivery of marine conservation in the UK is the *biotope*.
75 The idea is that suites of species commonly co-occur in locations with similar
76 environmental conditions. The collective term biotope encompasses both of these biotic
77 and abiotic elements. Attributes usually refer to the diversity, extent, distribution and
78 species composition of “biotopes”, and considerable effort has gone into defining and
79 describing a hierarchical habitat classification for UK marine waters (Connor et al. 2004)
80 to underpin their use in marine management. This classification has 6 levels, and is
81 compatible with the European Nature Information System EUNIS
82 (<http://eunis.eea.europa.eu/about.jsp>). Biotopes sit at level 5 (and sub-biotopes at level
83 6). Each is then nested within increasing levels, namely biotope complexes (Level 4),
84 habitat complexes (Level 3), broad habitat types (Level 5) and ultimately marine or
85 terrestrial environments (Level 1). Described categories in each level have associated
86 codes. For example, the biotope “Polychaetes, including *Paraonis fulgens*, in littoral fine
87 sand” has a EUNIS code A2.2311. A2 indicates the broad habitat type, littoral sediment,

88 A2.2 the habitat complex, littoral sand and muddy sand, A2.231 the biotope complex,
89 polychaetes in littoral fine sand, and finally the full code A2.231 indicates the particular
90 biotope within that complex. Within the UK similar categories are used, but with
91 different codes. Thus the code for this biotope is LS.LSa.FiSa.Po.Pful.
92 In this paper we describe a programme to monitor the conservation status of intertidal
93 sediments in the Isles of Scilly Complex SAC and recent results. We focus on issues that
94 arise through the application of the framework described above, and suggest possible
95 solutions to perceived problems.
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97 **2. St Martin's Flats monitoring**

98 *2.1 Methods*

100 2.1.1. The monitoring framework to be addressed

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102 Two Annex I habitats for which the Isles of Scilly Complex SAC has been designated are
103 "sandbanks which are slightly covered by seawater all the time" and "mudflats and
104 sandflats not covered by seawater at low tide". The conservation objective set by Natural
105 England is, "subject to natural change" to "maintain the mudflats and sandflats not
106 covered by seawater at low tide in favourable condition". There are no intertidal
107 mudflats on Scilly, and the biotopes comprising the intertidal sand habitat specified by
108 Natural England (2000) are:

109 *IMS.EcorEns*: Urchin *Echinocardium cordatum* and razor shell *Ensis* spp. in lower shore
110 fine sands and muddy sands;

111 *CGS.Ven*: Purple heart urchin *Spatangus purpureus* and bivalve community in lower-
112 shore sands; and

113 *LGS.Lan*: Sand mason worm *Lanice conchilega* in tidal-scoured lower-shore sands

114 The attribute to be measured is the "species composition of characteristic biotopes", the
115 measure is "presence, abundance and diversity of composite species from a range of sites,
116 measured once per reporting cycle" and the target that "composite species, abundance
117 and diversity should not deviate significantly from an established baseline, subject to
118 natural change".

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120 2.1.2. Field sampling and sample analysis

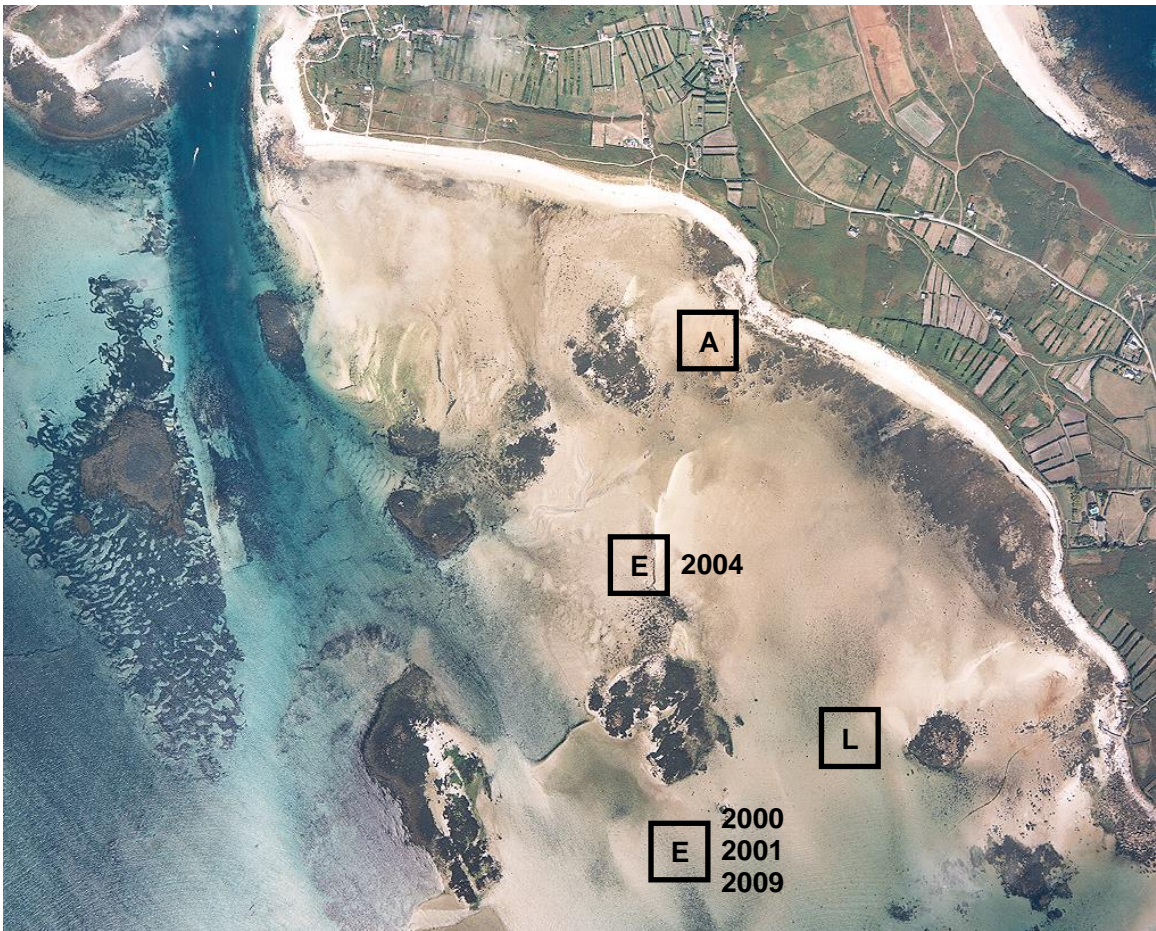
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122 Monitoring of the fauna of three biotopes that fall within the 'intertidal mud and sandflats'
123 feature began in August 2000. The three biotopes specified by Natural England (see
124 above) could not be distinguished, since the characterizing species of each were
125 frequently found together at a single site. Instead, three biotopes were defined
126 subjectively during an initial visual survey, based largely on physical and biogenic
127 sediment surface features. These were:

128 1) "*Arenicola*" Biotope: Fine sand with blackening close to the surface. Abundant
129 *Arenicola* holes and casts on sediment surface;

130 2) "*Ensis*" Biotope: Smoother, more waterlogged sand with evidence of live *Ensis* plus
131 large numbers of empty *Ensis* shells on sediment surface; and

132 3). "*Lanice/Echinocardium*" Biotope: Ripple-marked sand with sparse *Lanice* tubes (fans
133 apparently rather degraded) and *Echinocardium* burrow openings present.
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137 **Fig. 1** Aerial photograph of the St Martin's sedimentary shore indicating the areas
138 sampled for each of the 3 biotopes: L = *Lanice/Echinocardium*, E = *Ensis* and A =
139 *Arenicola* (Web colour, print B/W)
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141 10 replicate core samples, haphazardly distributed, were collected within a 20 m radius of
142 a central point: *Arenicola* 49°57'58.6"N 6°17'35.3"W; *Ensis* 49°57'33.78"N
143 6°17'34.1"W; *Lanice/Echinocardium* 49°57'40.0"N 6°17'17.0"W (Fig. 1). . For each
144 sample, a 0.1 m² stainless steel square corer was pushed into the sediment to a depth of
145 30 cm. Sediment within the core was then removed and gently sieved (puddled) over a
146 1mm mesh. The residue on the sieve was elutriated by resuspending the sediment in a
147 bucket of seawater that had been pre-filtered through a 0.5 mm sieve, and decanted onto a
148 1mm-mesh sieve. After 3 elutriations, the residue remaining in the bucket was carefully
149 hand-sorted and all organisms extracted and added to the elutriate. The sample was
150 preserved in 10% formalin.

151 In the laboratory, samples were washed free of formalin on a 0.5 mm mesh sieve and the
152 animals picked out under a binocular microscope. Individuals were identified to the
153 lowest practical taxonomic level using the most recent peer approved keys and literature
154 available. On St Martin's flats four species of the amphipod genus *Urothoe* were
155 recorded, but the positive identification of these species requires dissection and can be
156 very time-consuming, since several hundred specimens are present in the samples. There
157 is also some uncertainty regarding specific identification between different sample
158 analysts. Identification to genus level is less of a problem (dissection is not necessary) so
159 this group of species was been identified to genus level only. Species nomenclature
160 follows Howson & Picton (1997).

161 The survey was repeated in October 2004. The initial intention was to sample exactly the
162 same sites as were sampled in 2000. However, the original "*Ensis*" site sampled in 2000
163 was situated at Extreme Low Water of Spring Tides and was not uncovered by the tide
164 during that visit, despite this being the period of the lowest predicted tides for the latter
165 part of 2004. Accordingly an alternative site was selected (Fig. 1) which appeared to have
166 similar surface features to the original site, and five trial samples were collected here for
167 comparison. Additionally, four samples had been collected at the original "*Ensis*"
168 location in April 2001 using identical methodology but for a different study (Warwick et
169 al., 2006), and these samples are also used in the analysis of change. Most recently, the
170 survey was repeated in September 2009, when spring tides were sufficiently low that the
171 original "*Ensis*" site sampled in 2000 was exposed and could be resampled.

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173 2.1.3. Data analysis

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175 To address the measure "presence, abundance and diversity of composite species"
176 univariate measures of community structure and diversity [number of species (S), number
177 of individuals (N) and Simpson's evenness index ($1-\lambda'$)] were calculated for each sample.
178 Diversity profiles were visualised by plotting *k*-dominance curves, and species
179 accumulation plots were constructed based on the means of up to 999 permutations of the
180 sample ordering. Multivariate data analyses followed the methods described by Clarke
181 1993 and Clarke & Warwick, 2001 using the PRIMER (Plymouth Routines In
182 Multivariate Ecological Research) v.6 software package (Clarke & Gorley, 2006), and
183 using the Bray-Curtis similarity measure on square root transformed species abundance
184 data.

185 In addition, two other types of univariate measures were determined, and applied to the
186 time-series of data. AMBI (AZTI's Marine Biotic Index) was designed to analyse the
187 response of macrobenthic assemblages in European coastal waters to changes in
188 environmental quality (Borja et al., 2000, 2003). The species are classified into five
189 ecological groups depending on their sensitivity to environmental stress, and the index is
190 based on the relative abundances of individuals in each group. The index has become one
191 of the mainstays for the assessment of ecological status under the European Water
192 Framework Directive, and it was therefore considered appropriate to assess the ecological
193 status of the St Martin's Flats assemblages on these terms.

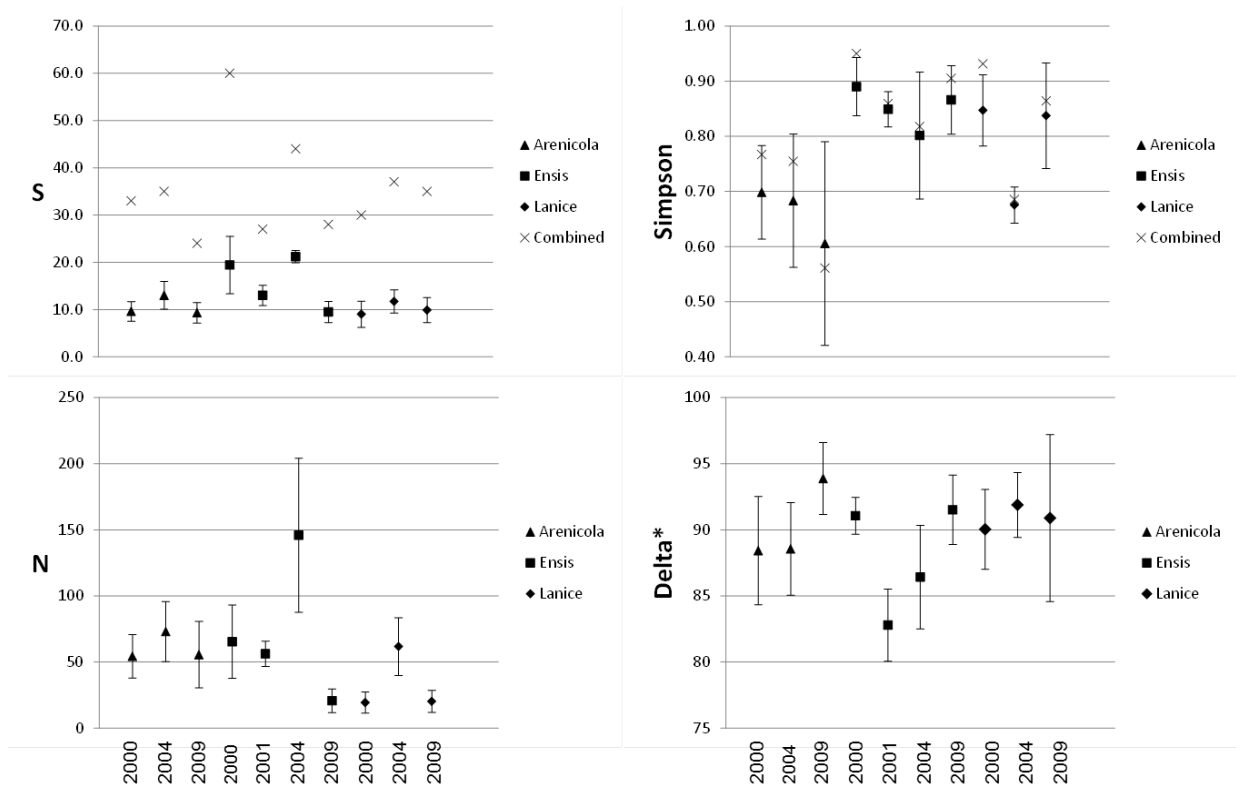
194 A group of biodiversity measures that are independent of species richness and sampling
195 effort, yet responsive to anthropogenic disturbance, considers the taxonomic relatedness
196 of species in the assemblage (Warwick & Clarke, 2001). It is well known that in impacted

197 assemblages of organisms the taxonomic spread of species is reduced, and in extreme
 198 cases they may be sibling species belonging to the same genus, or at least very closely
 199 related. Unimpacted assemblages, on the other hand, have a wider taxonomic spread and
 200 the species belong to many different genera, families, orders, classes and phyla. The
 201 measures used here are the average path length or taxonomic distance, traced through a
 202 taxonomic classification, between every pair of individuals (Δ), between every pair of
 203 individuals conditional on them being in different genera (Δ^*) and between every pair of
 204 species (Δ^+). A further measure (Δ^+) indicates the variability in the path lengths between
 205 species. The measures are independent of sample size or sampling effort, and are little
 206 affected by small variations in habitat type (Leonard et al., 2006). They can be used for
 207 data consisting simply of species lists and arising from unknown or uncontrolled
 208 sampling effort, which usually renders it impossible to read anything into the relative size
 209 of these lists. For Δ^+ there are permutation tests for the significance of departure from
 210 expectation under specific null hypothesis conditions.

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2.2. Results

2.2.1. Faunal diversity

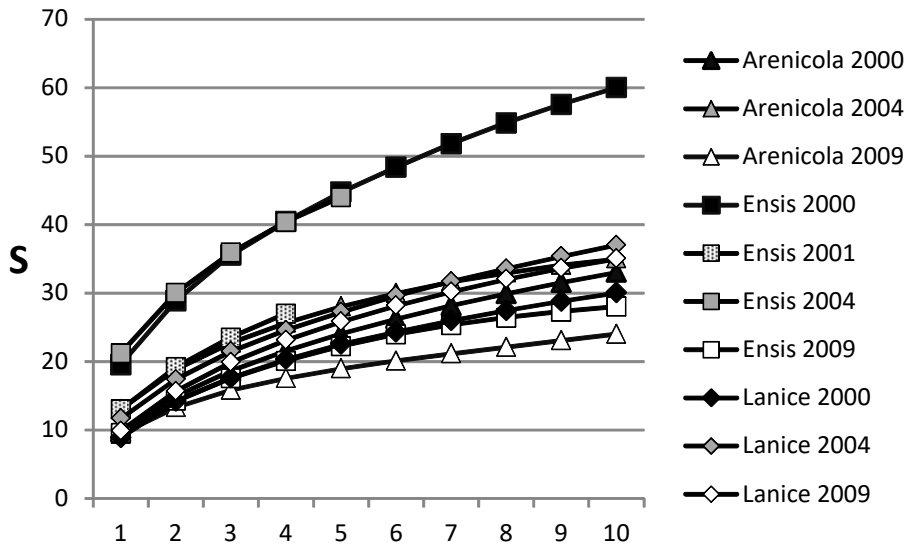


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218 **Fig. 2.** Univariate measures (S, number of species; N, number of individuals; Simpson,
 219 Simpson's evenness 1-λ' and; Delta*, taxonomic distinctness) from each biotope in each
 220 survey calculated from individual samples (mean ±s.d.). Values calculated from pooled
 221 samples are shown where these could differ markedly in behaviour from average values
 222 from replicates.

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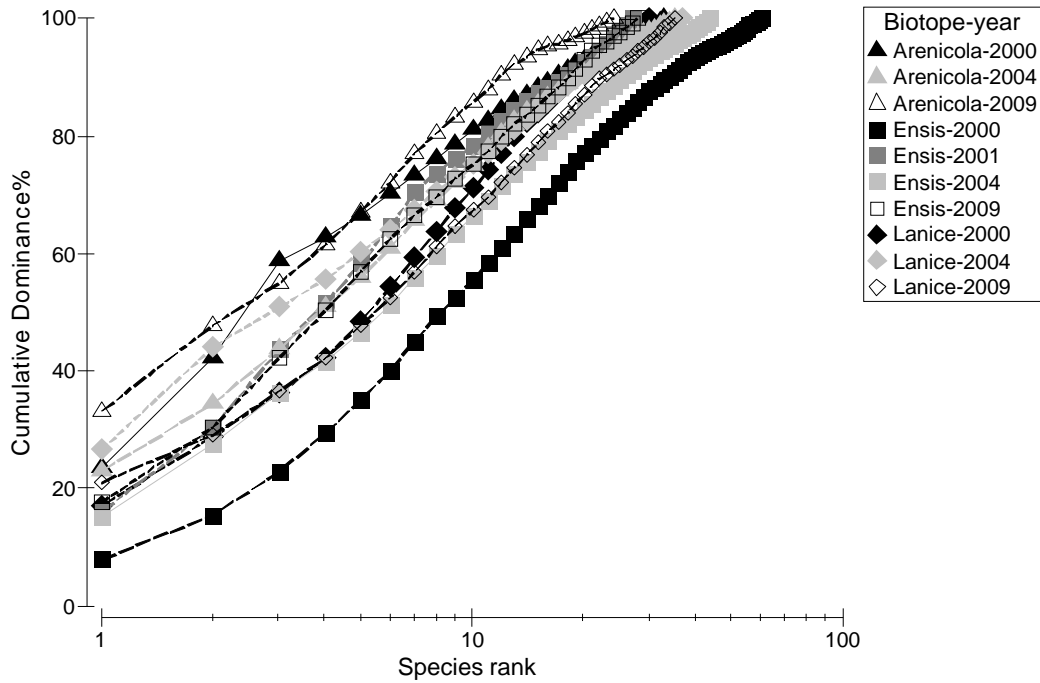
Values of a range of univariate measures (Figure 2) do not indicate consistent patterns within or among biotopes. Within-sample species richness is fairly consistent among samples from the *Arenicola* and *Lanice/Echinocardium* biotopes from different years, while richness in the *Ensis* biotope is more variable. Total richness, however, varies markedly across years in all biotopes. Abundance is highly variable across years in the *Ensis* and *Lanice/Echinocardium* biotopes, and less so in the *Arenicola* biotope. Within-sample evenness varies markedly across years in the *Lanice/Echinocardium* biotope, and less so in the others, while within-year variability is highest in the *Arenicola* biotope. Evenness calculated from combined samples from each survey tends to exacerbate among-year variability. Delta* shows clear changes among years in the *Ensis* biotope, less clear changes in the *Arenicola* biotope and little difference among years in the *Lanice/Echinocardium* biotope.



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Fig. 3. Species accumulation curves calculated from 1000 random permutations of replicate data from each biotope in each survey.

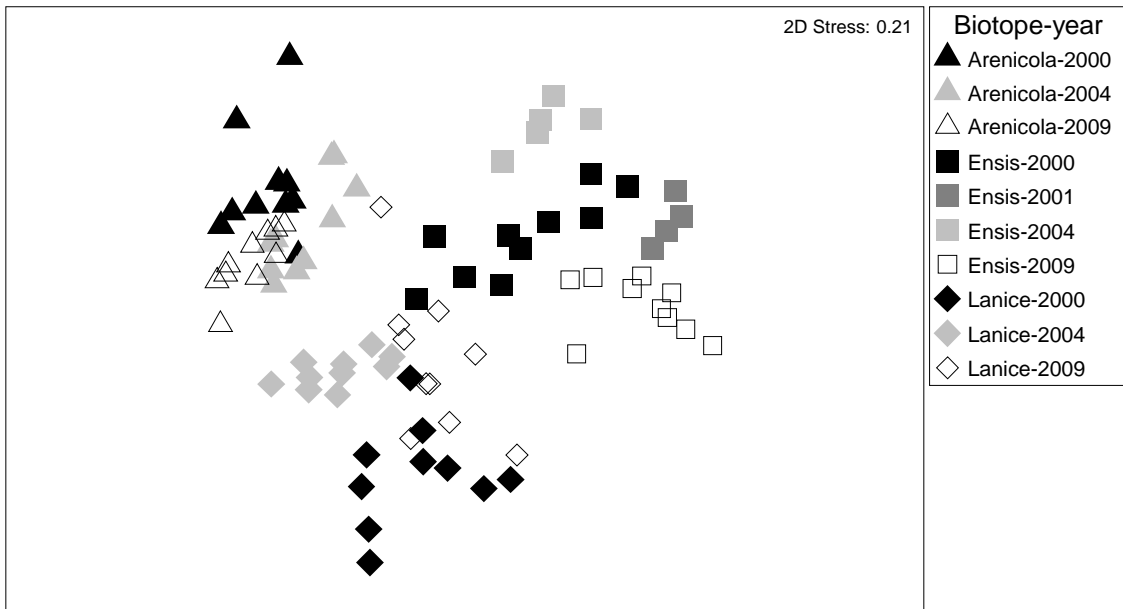
In view of the difference in sampling effort among years in the *Ensis* biotope, perhaps a better way of comparing richness is by examining species accumulation curves (Fig. 3). These plots allow sample sets with different numbers of replicates to be directly compared. They clearly separate two higher diversity sample sets, *Ensis* 2000 and *Ensis* 2004, and one lower, *Arenicola* 2009, from the remainder. Another graphical/distributional method, *k*-dominance curves (Fig. 4), indicate that diversity in combined samples from each survey was highest in the *Ensis* biotope in 2000, while differences among other combinations of biotope and year are less clear.



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Fig. 4. *k*-dominance plots calculated from pooled data from each biotope in each survey.

2.2.2. Community composition



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Fig. 5. MDS ordination of similarities among all samples, calculated using the Bray-Curtis coefficient on square-root transformed abundances.

261 Moving beyond analyses focusing on abundance and diversity, changes in species
 262 composition may be visualised using multivariate methods. An MDS ordination (Fig. 5)
 263 based on similarities among all samples clearly shows that the assemblages within each
 264 biotope remain distinct across years. The plot also indicates, however, that there are clear
 265 differences in species composition between different years within biotopes. Two-way
 266 SIMPER (Similarity Percentages) analysis was used to determine the species responsible
 267 for the similarity in the species composition among replicates from each biotope across
 268 all years, based on the root transformed species abundance data and the Bray Curtis
 269 similarity measure (Tables 1-3). In general, changes in species composition between
 270 years resulted from rather subtle changes in the relative abundances of a large number of
 271 species, rather than dramatic changes in abundance of a few dominants. A notable
 272 exception to this was the complete disappearance in 2009 of the distinctive cumacean
 273 *Apseudes latreilli* from the “Ensis” biotope, in which it had been very abundant in earlier
 274 years.

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Table 1 Percentage species contributions to the average similarity (46.96) among replicates across all years in the “*Lanice/Echinocardium*” biotope, ranked in order of importance, with a cut-off at 90%

<i>Species</i>	<i>Av. Abund</i>	<i>Av. Sim</i>	<i>Contrib%</i>	<i>Cum. %</i>
<i>Urothoe</i> spp.	3.23	15.97	34.01	34.01
<i>Ophelia rathkei</i>	1.72	6.43	13.69	47.71
<i>Travisia forbesii</i>	1.07	5.03	10.71	58.41
<i>Echinocyamus pusillus</i>	0.80	3.04	6.47	64.88
<i>Perioculodes longimanus</i>	0.77	2.76	5.88	70.76
<i>Echinocardium cordatum</i>	0.53	2.10	4.47	75.23
<i>Angulus tenuis</i>	0.51	1.58	3.37	78.60
<i>Amphioxus lanceolatus</i>	0.38	1.36	2.90	81.50
<i>Tellimya ferruginosa</i>	0.30	0.91	1.95	83.44
<i>Leptosynapta inhaerens</i>	0.23	0.82	1.75	85.19
Spionidae indet	0.32	0.81	1.71	86.91
<i>Dosinia exoleta</i>	0.35	0.80	1.70	88.61
<i>Nephtys caeca</i>	0.33	0.78	1.67	90.28

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Table 2 Percentage species contributions to the average similarity (49.92) among replicates across all years in the “*Ensis*” biotope, ranked in order of importance, with a cut-off at 90%

<i>Species</i>	<i>Av. Abund</i>	<i>Av. Sim</i>	<i>Contrib%</i>	<i>Cum. %</i>
<i>Dosinia exoleta</i>	1.93	8.40	16.84	16.84
<i>Ehlersia cornuta</i>	2.03	5.55	11.12	27.96
<i>Glycera lapidum</i> complex	1.30	5.22	10.46	38.42
<i>Notomastus latericeus</i>	1.83	3.97	7.95	46.38
<i>Apseudes latreillii</i>	1.87	3.31	6.64	53.01
<i>Aonides oxycephala</i>	1.68	2.94	5.90	58.91
<i>Urothoe</i> spp.	1.28	2.80	5.61	64.52
<i>Echinocardium cordatum</i>	0.53	2.26	4.53	69.05
<i>Echinocyamus pusillus</i>	0.99	1.94	3.89	72.93
<i>Leptosynapta inhaerens</i>	0.62	1.52	3.05	75.98

<i>Amphioxus lanceolatus</i>	0.76	1.49	2.99	78.97
<i>Moerella pygmaea</i>	0.69	1.37	2.75	81.72
<i>Lutraria lutraria</i>	0.29	0.84	1.69	83.41
<i>Perioculodes longimanus</i>	0.54	0.83	1.66	85.06
<i>Iphinoe trispinosa</i>	0.67	0.79	1.58	86.64
<i>Ensis arcuatus</i>	0.41	0.70	1.40	88.04
<i>Gari depressa</i>	0.42	0.69	1.39	89.43
<i>Mediomastus fragilis</i>	0.48	0.47	0.95	90.38

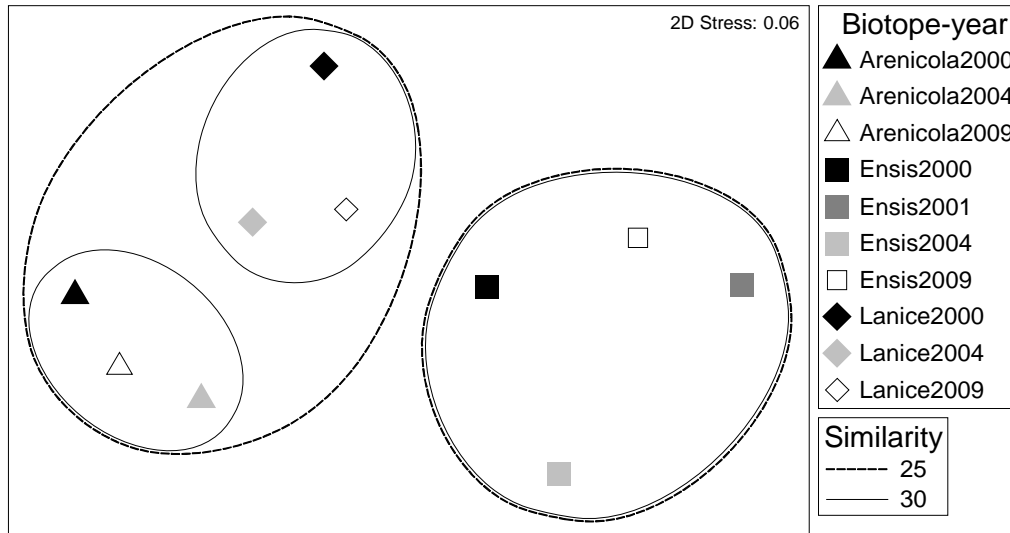
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Table 3 Percentage species contributions to the average similarity (56.19) among replicates across all years in the “*Arenicola*” biotope, ranked in order of importance, with a cut-off at 90%

Species	Av. Abund	Av. Sim	Contrib%	Cum. %
<i>Urothoe</i> spp.	4.90	18.71	33.30	33.30
<i>Scoloplos armiger</i>	3.21	13.76	24.49	57.79
<i>Malacoceros fuliginosus</i>	1.01	4.13	7.34	65.13
<i>Nephtys hombergii</i>	0.76	2.92	5.20	70.33
<i>Notomastus latericeus</i>	1.13	2.82	5.01	75.35
<i>Euclymene oerstedii</i>	0.71	1.53	2.73	78.07
<i>Arenicola marina</i>	0.68	1.28	2.29	80.36
<i>Spio filicornis</i>	0.57	1.16	2.06	82.42
<i>Pygospio elegans</i>	0.47	1.05	1.87	84.29
<i>Sphaeroma serratum</i>	0.41	1.00	1.77	86.06
<i>Crangon crangon</i>	0.47	0.98	1.74	87.80
<i>Angulus tenuis</i>	0.41	0.83	1.47	89.27
<i>Perioculodes longimanus</i>	0.40	0.69	1.23	90.50

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An alternative multivariate approach is to ask whether there is any evidence for differences in average (or total) species composition among surveys. An MDS based on pooled samples from each survey (Fig. 6) indicates consistency in composition within biotopes as surveys are grouped together, with a similarity >30. The contours in Fig. 6 show samples that cluster together at given levels of similarity within a corresponding cluster analysis. A corresponding Similarity Profiles (Simprof) test shows no evidence for multivariate structure within the clusters grouped at 30% similarity.



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Fig. 6. MDS of similarities among averaged data from each biotope in each survey, derived from Bray-Curtis similarities and square-root transformed abundances.

2.2.3. Statistical significance

Differences in variability and sampling effort present problems in the context of applying standard statistical methods, such as analysis of variance, to determine whether differences in univariate measures among biotopes and across years are in some sense significant. There is an alternative robust non-parametric testing framework available, which is to calculate differences in a measure among samples and to analyse the resulting distance matrix using ANOSIM. ANOSIM can also be used to test for differences in k -dominance curves among groups of samples, by calculating distances between curves, and also in its more familiar application to analyse for differences in multivariate community structure using a resemblance matrix. Here we use the Bray-Curtis resemblances among samples calculated from square-root transformed abundances. A summary of results (Table 4) clearly shows that most methods, univariate, graphical/distributional and multivariate, indicate statistically significant differences among all combinations of biotopes and years. Simprof, an alternative approach which tests for multivariate structure without recourse to an a priori defined group structure, does not detect any difference in community structure between the pooled samples from the *Lanice* and *Arenicola* biotopes, but does between samples from the *Ensis* biotope and the others. In other words, samples grouped together at a similarity of >25 in Fig. 6 form 2 distinct groups within which there is no statistical support for further subdivision.

Table 4 Summary of 1-way Anosim tests for differences between biotopes and surveys. Entries indicate tests with $p < 0.05$, inferring differences. Response variables are indicated as: S, number of species; N, number of individuals; E, Simpson's evenness index; D, taxonomic distinctness Δ^* ; k, k -dominance curves; B, Bray-Curtis similarities calculated using root-transformed abundances.

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Arenicola 2004	S, k, B	...								
Arenicola 2009	E, D, k, B	S, D, B	...							
Ensis 2000	S, E, D, k, B	S, E, D, k, B	S, E, D, k, B	...						
Ensis 2001	S, E, k, B	D, B	S, D, k, B	D, B	...					
Ensis 2004	S, N, E, k, B	S, N, k, B	S, N, D, k, B	N, D, B	S, N, k, B	...				
Ensis 2009	N, E, D, k, B	S, N, E, k, B	N, E, k, B	S, N, k, B	S, N, D, B	S, N, D, k, B	...			
Lanice 2000	S, N, E, k, B	S, N, E, k, B	N, E, D, k, B	S, N, k, B	N, D, B	S, N, D, k, B	B	...		
Lanice 2004	S, E, D, k, B	E, B	E, k, B	S, E, k, B	E, D, k, B	S, N, E, D, k, B	N, E, k, B	N, E, k, B	...	
Lanice 2009	N, E, k, B	N, E, k, B	N, E, k, B	S, N, D, k, B	N, D, B	S, N, k, B	B	B	N, E, k, B	
	Arenicola 2000	Arenicola 2004	Arenicola 2009	Ensis 2000	Ensis 2001	Ensis 2004	Ensis 2009	Lanice 2000	Lanice 2004	

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2.2.4. Alternative approaches

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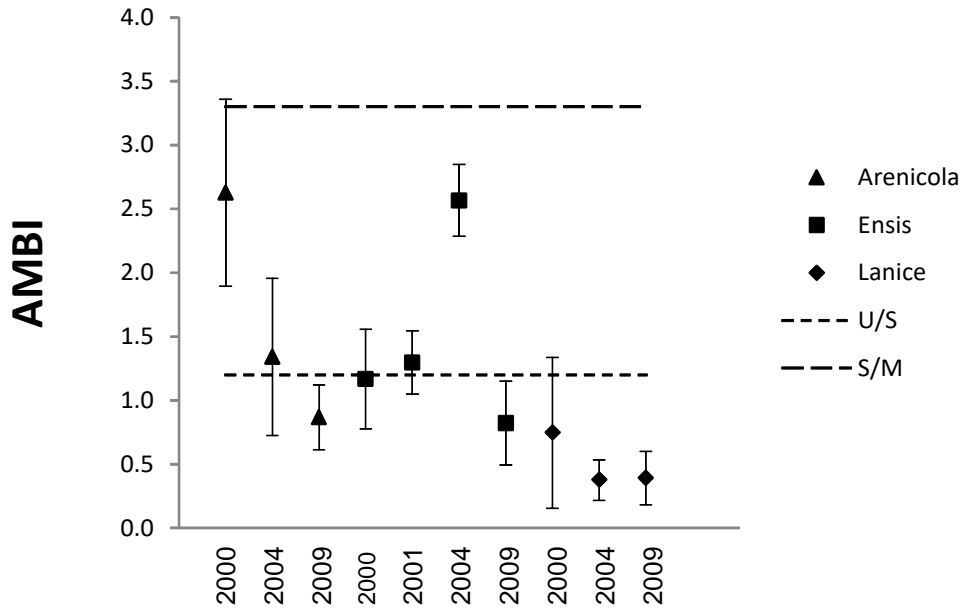
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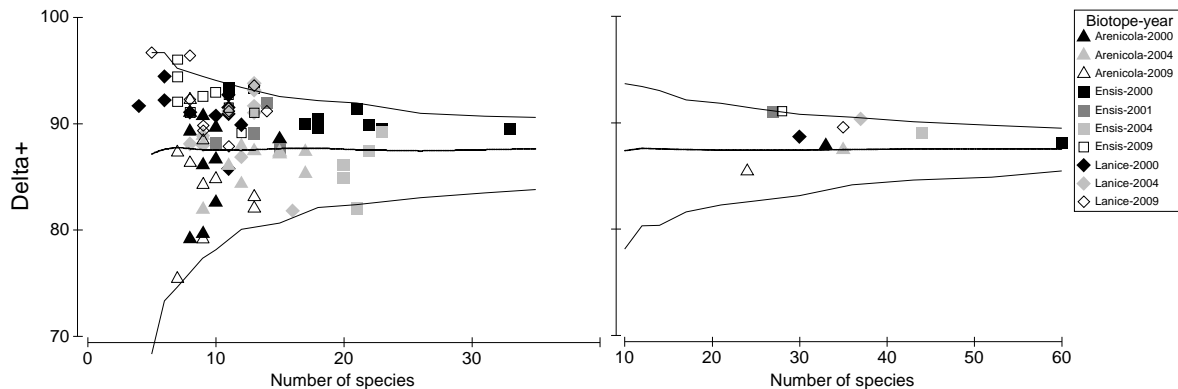
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In the context of the monitoring framework under discussion, the relevant target is that “composite species, abundance and diversity should not deviate significantly from an established baseline, subject to natural change”. The issue with all of the methods discussed so far is that comparisons are relative. A measure in one biotope in one year can only be compared to the same measure calculated from comparable survey data in another biotope and/or another year. Rather than comparing surveys among years, an alternative approach is to apply measures that have, in some sense, expected values reflecting differences in environmental quality. AMBI is a measure of the average pollution tolerance of an assemblage based on the observed pollution tolerances of species within the assemblage. A low score indicates that most individuals belong to species known to be intolerant of pollution, while a high score indicates that most individuals belong to species highly tolerant of pollution. Based on survey data from a large number of sites in the NE Atlantic numerical limits for AMBI have been selected to indicate differences in ecological status, so ecological status may be assigned based on single samples. Based on average values from the replicate data (Fig. 7), the majority of surveys indicate undisturbed assemblages, dominated by individuals from species which are considered to be intolerant of pollution. The *Arenicola* biotope in 2000 and 2004 and the *Ensis* biotope in 2001 and 2004 fall into the slightly disturbed category, indicating the presence of individuals from species which have some pollution tolerance. All surveys, however, indicate that the environment is in good status or better. While there is a potential for values calculated from pooled data to show slightly different patterns, for these data differences in AMBI calculated directly from pooled data and averages from replicates were small (<0.1 units) and in no case altered the conclusions to be drawn.



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 368 **Fig. 7.** Values for AMBI (mean \pm 1 s.d.) from samples in each biotope in each survey.
 369 Lower values indicate better ecological state, with U/S indicating the
 370 ‘undisturbed/slightly disturbed’ boundary and S/M the ‘slightly/moderately disturbed’
 371 boundary. Values from pooled samples track mean values very closely, so they are not
 372 shown.
 373

374 A different approach is to look at the average relatedness of species in assemblages, using
 375 Δ^+ . A combination of observation and theory suggests that under unimpacted conditions
 376 the species observed at a particular time or place will be a random subset of the species
 377 that may occur there, while under the influence of environmental stress the species
 378 observed will tend to become more closely related to each other. Using a list of all
 379 species recorded in all surveys to date as the master list, results (Fig. 8) indicate that most
 380 samples fall within expectation. In other words, there is no evidence that species are
 381 more closely related to each other than expected. This is true of both individual samples
 382 and of pooled samples from each survey.
 383



384
 385 **Fig. 8.** Funnel plots indicating how observed values of taxonomic distinctness calculated
 386 from species lists (Δ^+) plotted against the number of species in each list, relate to values

387 corresponding to species being picked at random from the complete list of species
388 collected from St Martin's Flats. Lines indicate the expected mean Δ^+ and 95% of
389 observations are expected to lie between the upper and lower bounds. Individual replicate
390 samples on left, pooled samples on right.

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393 2.3. Discussion

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395 2.3.1. Faunal changes over time

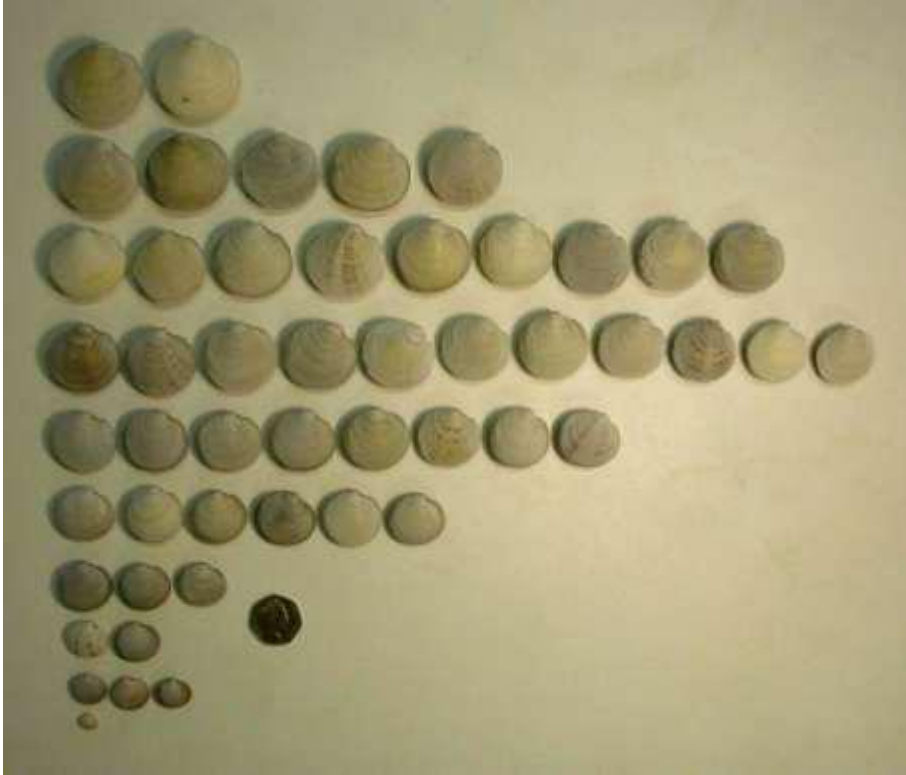
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397 A subjective impression of the surface features of the three sites suggested that in the
398 "*Lanice/Echiocardium*" biotope there were fewer feeding fans of the sand-mason worm
399 *Lanice* in 2009 than in previous years and in the "*Ensis*" biotope there were fewer dead
400 razor shells on the sediment surface and less evidence of the presence of live specimens
401 (i.e. squirting water when disturbed).

402 Detailed analysis shows that, although the species composition of each biotope has
403 changed significantly, the biotopes have retained their integrity between 2000 and 2009.
404 Each biotope in 2009 was closer in composition to that same biotope in 2000 and 2004
405 than to any other biotope. Diversity profiles (particularly in terms of species
406 accumulation plots) were unchanged for the "*Lanice/Echinocardium*" and "*Arenicola*"
407 biotopes, but for the "*Ensis*" biotope diversity was much lower in 2009 than in 2000, the
408 only other strictly comparable year in terms of sampling location and number of
409 replicates. There were also larger changes in the species composition of the "*Ensis*"
410 biotope between years than in the other two biotopes (Figs 5 & 6). This biotope is a more
411 physically dynamic habitat than the other two, as evidenced by the coarseness of the
412 sediment, and is therefore more likely to be subject to short term fluctuations in species
413 composition and diversity. Small short-lived species are likely to fluctuate in abundance
414 from year to year, as exemplified by the disappearance of the cumacean *Apseudes latreilli*
415 from this biotope. Some large species that can live for many years may have regular
416 recruitment in each year and establish temporally stable populations, while others may
417 have exceptionally successful recruitment in some years but recruitment failures in
418 others. An example of the former is the clam *Dosinia exoleta*, which was represented in
419 the 2009 samples by about 10 year-classes of various strength (Fig. 9). On the other hand
420 another large bivalve, the razor shell *Ensis arcuata*, large specimens of which had
421 initially been used to define this biotope, had virtually disappeared in 2009, while very
422 large and conspicuous specimens of the bivalve *Lutraria lutraria* were present (Fig. 10).
423 This species was absent in the 2000 samples and the specimens all appeared to be of the
424 same age (~8 years) with no younger individuals present, suggesting settlement soon after
425 2000 but with no subsequent recruitment.

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Fig. 9. Specimens of the clam *Dosinia exoleta* from the “*Ensis*” biotope in 2009, arranged in year classes and indicating successful recruitment each year. (Web colour, print B/W)



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Fig. 10. Specimens of the clam *Lutraria lutraria* from the “*Ensis*” biotope in 2009, indicating a single year-class with no recruitment in recent years. (Web colour, print B/W)

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2.3.2. Biotope identification

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To recap, within the monitoring framework which aims to underpin marine conservation in the UK, a number of steps need to be taken to assess whether conservation objectives are being achieved. Characteristic biotopes must be identified, and within them composite species, abundance and diversity should not deviate significantly from an established baseline, subject to natural change.

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It is immediately apparent that the classification system erected for marine biotopes in the UK does not include the biotopes present on St Martin’s flats. The point of interest is, then, whether it should, or could. It is not our goal, here, to critique the hierarchy in its entirety, or to discuss its general utility in its current form. We do, however, question some of the assertions on which it is based. The idea that, given identical environmental conditions (and sufficient time), an identical association of species should develop, underpinned much of the development of community ecology in the first half of the 20th century, building on the work of Francis Clements. In what is still, probably, the most insightful review of marine benthic ecology, Thorson (1957) implicitly considers the consequences of such a view of community development on classification schemes for marine benthic communities. Although from the 1950s onwards a strict Clementsian view of ecological development has generally been replaced by a Gleasonian view of ecology, in which individual species’ responses underpin apparent associations and a stochastic element is important, Thorson’s (1957) views are still highly relevant. Among these is that the “level bottom lacks the numerous “microlandscapes” (exposed or protected rocks, associations of different plants, holes, crevices etc.), each with a special microclimate, so characteristic of epifaunal environments”. It is worth noting that the

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471 biotope classification was initially devised using, primarily, data gathered on the shore
472 and using SCUBA with a focus on epifaunal environments. Thorson's view is that soft-
473 bottom habitats are primarily driven by hydro-physical factors operating over large areas,
474 which determine sediment composition, food supply and larval settlement, so while it
475 may be possible to split epifaunal environments into "micro-units", a "similar splitting of
476 level-bottom communities should be avoided" and "it seems reasonable, therefore, to
477 divide the animal communities in accordance with these large natural bottom units".
478 None of the apparent biotopes on St Martin's Flats corresponds exactly with any of those
479 currently classified by the JNCC (Connor et al., 2004). This is either because these
480 sedimentary habitats are unlike any of those surveyed to construct the classification, or
481 because the classification itself is too discriminating, as Thorson's (1957) work would
482 suggest. In fact, it is likely that both factors have an influence. Matches can be found
483 with levels 2 and 3 of the biotope classification which relate to the physical characters of
484 the habitat, but at level 4 and higher the faunistic composition begins to comprise part of
485 the definition and no exact matches can be found. An online supplementary table lists
486 potential candidates from the National Biodiversity Network database for level 2 Littoral
487 sediment (LS) and Sublittoral sediment (SS) habitats previously recorded from Scilly.
488 The latter were considered because, as noted by early naturalists (Carus 1850), there are
489 many species that occur intertidally on Scilly that are only found in deeper water
490 elsewhere in Britain. Many species characterizing level 5 habitats (biotopes) that belong
491 to the level 2 Sublittoral sediment habitat in the JNCC classification were found
492 intertidally in the surveys of St Martin's Flats. Holme (1961) listed a number of bivalve
493 molluscs from other locations on Scilly that fall into this category, as do the crinoid
494 *Antedon bifida*, the conspicuous orange seven-armed starfish *Luidia ciliaris* and the
495 cephalochordate *Branchiostoma lanceolatum* (the latter being frequent on St Martin's
496 Flats). Harvey (1969) makes a number of suggestions as to the causes of this
497 phenomenon. The relative scarcity of near zero temperatures may permit animals to come
498 up into the littoral, as may the negligible lowering of salinity compared to other places
499 where lower salinities might deter some species, especially echinoderms, from littoral
500 life. The phenomenon is not confined to the macrobenthos. Hummon and Warwick
501 (1990) found several meiobenthic interstitial gastrotrich species in sandy beaches of
502 Scilly that elsewhere only occurred sublittorally. They suggested that an additional
503 possible explanation for this was the angularity of the sand grains derived from granite,
504 which were tightly packed and restricted drainage from the beach at low tide, resulting in
505 an interstitial environment no different from the sublittoral. It is clear from the online
506 supplementary table that a large number (nearly half) of the 97 records from Scilly
507 provide an uncertain match with a previously recognised biotope, in which cases attempts
508 to ascribe them to such biotopes seems inappropriate. Furthermore, only 65 of these were
509 identified as biotopes (level 5), of which 35 were uncertain matches, the remainder being
510 identified either at level 4 (biotope complexes, 21 records) or level 3 (habitat complexes,
511 7 records).

512 Nevertheless, at least two or three recognisable associations of species are present on St
513 Martin's Flats, and more extensive mapping might reveal more. If these were to be
514 formalised for the purposes of inclusion in a wider classification the biotope names
515 initially ascribed to two of these associations for the purposes of this study,
516 "*Lanice/Echinocardium*" and "*Ensis*", should not be retained since *Lanice* and *Ensis* are

517 no longer features of them, or at least have been shown to be inconsistent indicators. A
518 more realistic definition of these assemblages could be gained from examination of the
519 species that *consistently* make a substantial contribution to the Bray Curtis similarity
520 among samples collected from each location (Tables 1-3). Candidate species that *typify*
521 that assemblage should be found at a consistent abundance throughout, so the standard
522 deviation of their contribution is low, and the ratio of Similarity/SD is high. For the
523 “*Ensis*” biotope there is a clear candidate for the characterising species: the clam *Dosinia*
524 *exoleta* makes the greatest contribution to the similarity among replicates and is the most
525 consistent, with the highest Similarity/SD ratio (Table 2). It is also large and easily
526 recognisable (Fig. 9). For the “*Arenicola*” biotope (Table 3) the greatest contribution to
527 the similarity among samples is made by *Urothoe* spp., but these amphipods also make
528 the greatest contribution to the “*Lanice/Echinocardium*” biotope. The next most
529 important contribution is made by the polychaete *Scoloplos armiger*, which is unique to
530 this assemblage and is also the most consistent, and it is also appropriate to retain the
531 lugworm *Arenicola marina* as an assemblage-defining species in view of its large size
532 and the consistently clear indications of its presence from surface features (casts and
533 burrows). Thus this could be designated the “*Arenicola/Scoloplos*” assemblage. The
534 original “*Lanice/Echinocardium*” biotope is the most problematic, since many of the
535 species that contribute to the similarity among samples are also found at the other two
536 sites. However, two opheliid polychaetes *Ophelia rathkei* and *Travisia forbesii* make the
537 second and third highest contributions to inter-sample similarity (Table 1) and are unique
538 to this assemblage, so this could be termed the “*Echinocardium/Opheliid polychaetes*”
539 assemblage. The term “assemblage” rather than “biotope” is used here for the purposes
540 of this study, rather than adding to the plethora of named biotopes that already exist and
541 which are constantly being added to with each new area investigated.
542 Of course, an alternative view could be that despite differences between different areas of
543 the Flat these do not represent separate biotopes, but variation between different places
544 driven by differences in tidal height and exposure. An objective method, such as
545 Simprof, reinforces this idea, providing statistical support only for separating the *Ensis*
546 biotope, from the extreme lower shore, from the other two (Fig. 6).

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548 2.3.3. Favourable condition

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550 The targets for the benthic fauna are that composite species, abundance and diversity
551 “should not deviate significantly from an established baseline, subject to natural change”.
552 The obvious problems here are defining the baselines, distinguishing between natural and
553 anthropogenic change and determining how much change constitutes significant
554 deviation. The question also arises as to whether significance is a biological, social or
555 statistical construct.

556 Multivariate analyses have shown that, for each of the three study areas, there have
557 been statistically significant changes in species composition between years. There is no
558 reason to suppose that these changes are not natural, and with a naturally fluctuating
559 baseline it is not easy to determine what degree of change is acceptable and how this
560 could be measured. Similarly, a reduction in species diversity in 2009 for the “*Ensis*”
561 biotope, compared with earlier years, is difficult to assess unless the range of natural
562 variation to be expected in such a habitat is known, and sampling on only three occasions

563 cannot establish this. The ecological condition determined by the AMBI score is based on
564 a global comparison with other areas. All three biotopes were in the “undisturbed”
565 category in 2009, and future change into a category worse than has been found any of the
566 ealier surveys could, in future, be taken as an unfavourable condition needing further
567 investigation.

568 Taxonomic distinctness measures of biodiversity are, unlike species richness
569 measures, relatively insensitive to small natural changes in habitat but are sensitive to
570 anthropogenic disturbance (Leonard et al., 2006). For taxonomic distinctness indices
571 based on simple species lists (presence or absence of species) there is a potential
572 framework within which these measures can be tested for departure from expectation (see
573 Warwick & Clarke, 2001). This envisages a master list or inventory of species
574 encompassing the appropriate region/biogeographic area, from which the species found at
575 one locality can be thought of as drawn. For example, Fig. 8 uses the complete faunal list
576 for St Martin’s Flats in all biotopes and years. The species complement at any particular
577 biotope and year can be compared with the master list, to ask whether the observed subset
578 of species is representative of the biodiversity expressed in the full species inventory.
579 Clearly, such a comparison is impossible for species richness since the list at one location
580 is automatically shorter than the master list. However, the key point here is that average
581 taxonomic distinctness (Δ^+) of a randomly selected sublist does not differ, in mean value,
582 from AvTD for the master list, and reductions from this level can be interpreted as loss of
583 biodiversity. Furthermore, there is a natural testing framework for how large a decrease
584 (or increase) from expectation needs to be, in order to be deemed statistically significant.
585 For an observed set of m species at one location, sublists of size m are drawn at random
586 from the master inventory, and their AvTD values computed. From, say, 999 such
587 simulated sublists, a histogram can be constructed of the expected range of Δ^+ values, for
588 sublists of that size, against which the true Δ^+ for that locality can be compared. If the
589 observed Δ^+ falls outside the central 95% of the simulated Δ^+ values, it is considered to
590 have departed significantly from expectation. The construction of these 95% probability
591 intervals can be repeated for a range of sublist sizes ($m = 10, 15, 20, \dots$) and the resulting
592 upper and lower limits plotted on a graph of Δ^+ against m . When these limit points are
593 connected across the range of m values, the effect is to produce a funnel plot (such as
594 seen in Fig. 8). The real Δ^+ values for a range of observational studies are now added to
595 this plot, allowing simultaneous comparison to be made of distinctness values with each
596 other and with the expected limits. For the St Martin’s flats biotopes, measured values of
597 Δ^+ all fall within the 95% confidence limits of the simulated null distribution based on
598 random samples from the master list (Fig. 8), suggesting that biodiversity in these terms
599 does not depart from expectation. If biotopes fall outside these 95% confidence limits in
600 future, an unfavourable condition would be indicated.

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602 2.3.4. Temporal variability

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604 In the specific case of St Martin’s Flats we have addressed the question of the extent of
605 natural variation that should be accounted for when setting conservation objectives. In a
606 much wider study, to be reported elsewhere, we searched for raw data relevant to features
607 which could be the target of marine conservation objectives from anywhere on the
608 continental shelf of the North-East Atlantic. None had the combination of spatio-

609 temporal coverage and relevance required for them to be used to set, quantitatively, levels
610 of natural variation which could be built into robust and defensible conservation
611 objectives. This should not be a surprise. Gray and Elliott (2009) identify three general
612 patterns of temporal variability in marine benthic systems. Some species tend to maintain
613 population numbers relatively constant through time and may be said to be persistent;
614 many organisms undergo repeatable cycles, which may be annual or longer term with
615 periods from 6-7 to >30 years; there may be changes in response to longer-term processes
616 which may or not be cyclical such as variation in the NAO. These patterns may be
617 regarded as stable as changes are to some extent predictable, but may only be understood
618 if we have monitoring data at the appropriate temporal and spatial scales. Populations
619 change with variable recruitment (and the processes underlying that variability): some
620 species recruit regularly, such as *Dosinia exoleta* in the St Martin's Flats example, while
621 others have highly successful pulses of recruitment followed by long periods with no
622 recruitment at all, such as *Lutraria lutraria* in the St Martin's Flats example. Whether the
623 latter may be considered stable or not depends on the repeatability of the cycles and the
624 scale at which variation is considered. Gray and Elliott (2009) state that insufficient
625 information is available on this, and go on to say "In fact, so little data is available on
626 long-term cycles and variations in recruitment that the patterns described above may in
627 time prove not to be typical at all. Understanding recruitment variability and the factors
628 causing that variability is one of the central problems in understanding long-term
629 fluctuations in benthic communities." It should also be noted that not only species and
630 populations exhibit variation on many temporal scales. Assemblages also do, and most
631 assemblages are in some form of dynamic equilibrium. Thus repeat surveys of the same
632 place might detect very similar communities, but as in the St Martin's Flats example, they
633 will not be identical. They might detect very different communities which form parts of a
634 natural successional cycle (e.g. mussels, barnacles or algae, on rocky shores). In terms of
635 setting objectives, consideration needs to be made of the degree of change that might be
636 considered trivial, as opposed the degree if change that might be of concern. In such a
637 framework, however, percent change is unlikely to be an applicable measure.
638 The question then is: how to take account of natural variation within conservation
639 objectives without having a clearly defensible method for setting numerical limits? The
640 simplest is to phrase objectives in a way that acknowledges that variation occurs, while
641 allowing expert judgement to play a role in determining the cause and consequences of
642 that variation. Conservation objectives consider two main components of features: extent
643 and status (or quality). While it may be difficult to do in practice, determining changes in
644 the extent of a feature presents little intellectual challenge unless the feature is poorly
645 defined. Setting of objectives relating to conservation status, however, is more
646 challenging in a quantitative context. The nature, direction, degree and interpretation of
647 changes depend, critically, on how status is defined and determined. For example,
648 Warwick et al. (2002) demonstrated that different measures of diversity, applied to the
649 same dataset, led to very different interpretations of change in the community under
650 consideration. Measures of abundance and species richness, the types of measures
651 describing amounts and therefore amenable to incorporation in a numerical framework
652 based on percent change, were uninformative and varied considerably. Other measures
653 showed a clear step-change in community structure which could be interpreted as positive
654 (improvement) or negative (decline) depending on the underlying conceptual model

655 being applied. A classic example is the failure of the monitoring of Norwegian oil
656 platforms to detect change, when using simple numerical treatments of monitoring data
657 (Gray et al. 1990). Application of alternative numerical methods to the same data
658 showed that conservation objectives (no change beyond 500m from the rigs) were not
659 being complied with, and led to major changes in the industry and the way in which
660 monitoring was carried out. It seems sensible, therefore, to focus numerical ranges and
661 limits for conservation objectives on aspects of features that may be described in
662 appropriate terms. An objective of the form “diversity of species should not decline by
663 more than 10%” is unlikely to be useful, unless there is a clear expectation that such a
664 decline may occur and may be informative. A further consideration is that of statistical
665 power. Setting a conservation objective with numerical bounds implies that changes may
666 be detected accurately. Several benthic studies (e.g. Rogers et al. 2006) have shown that
667 the degree of sampling effort required for the detection of small (<10%) changes is
668 prohibitive (100s to 1000s of samples being required) and only if changes in the order of
669 50-75% are to be detected with any degree of certainty does the required sampling effort
670 begin to be practical. On the other hand, an objective of the form “good conservation
671 status must be maintained” leaves the door open for sensible data collection, analysis and
672 interpretation.

673

674 **3. Conclusions**

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676 Anthropogenic threats to marine biodiversity are many and varied, and operate on spatial
677 and temporal scales ranging from local short-term pollution incidents or coastal
678 developments to regional long-term effects of fishing activities, eutrophication, climate
679 change or the effects of introduced species. Because most traditional biodiversity indices
680 based on species richness are strongly affected by natural environmental variability,
681 distinguishing between natural and anthropogenic changes is generally recognised as the
682 most difficult challenge facing biodiversity monitoring. ICES (2002) has observed the
683 inappropriateness of the ‘pristine state’ as a default reference point against which the
684 biodiversity of potentially impacted sites can be evaluated. Nevertheless, there is a
685 requirement to assess “good ecological condition / favourable condition” for designated
686 sites. We suggest above that setting limits on natural variability is almost always
687 impractical, or at least requires subjective judgement which is often indefensible.
688 Disentangling the drivers of biodiversity change adequately has required experiments in
689 which environmental variables can be manipulated individually in a controlled way;
690 generally impractical for routine monitoring programmes and of dubious relevance to the
691 real world. The advantage of taxonomic distinctness is that variability in biodiversity due
692 to natural environmental factors generally falls within a predictable range (Leonard et al.
693 2006) , based on the expectation of random selection from a regional species pool. This
694 expectation then becomes the baseline against which biodiversity change is determined,
695 instead of relying on historical time-series data. Anthropogenic influences modify this
696 pattern, such that biodiversity falls below the predicted range. The taxonomic distinctness
697 index is easy to measure (relying on simple species lists rather than quantitative data) and
698 it has been shown to be appropriate as an indicator of the effects on biodiversity of
699 anthropogenic events over a range of spatial and temporal scales (Leonard et al. 2006). It

700 also explicitly addresses issues of conservation, protection and enhancement of biological
701 diversity advocated by the global Convention on Biological Diversity.

702 Another alternative to setting limits of natural variability based on time-series data at a
703 particular site is to examine the spatial variability in habitat quality over the geographical
704 range that that habitat occupies. Many data exist, but they are difficult to use in
705 quantitative comparisons. With the taxonomic distinctness index the concept of spatial
706 reference sites is replaced by the concept of a “reference condition”, i.e. the null
707 hypothesis that the species present are structured as if they are a random selection from
708 the regional species pool. This could enable the establishment of a reference condition in
709 a region that was entirely impacted to some degree, and where no appropriate reference
710 sites are available. Thus, the desired “favourable condition” for an interest feature in an
711 SAC might not necessarily be the condition it was in at the time it was designated. The
712 application of AMBI (AZTI’s Marine Biotic Index) is a means of comparing the
713 ecological status of an assemblage of species based on their sensitivity to pollution and
714 disturbance at a wide range of reference sites, and the AMBI score is an additional means
715 of assessing favourable condition irrespective of temporal variability in community
716 composition and diversity.

717 Generally faunistic surveys only determine species abundances, which limits the number
718 of techniques available for assessing ecological condition. Some consideration might also
719 be given to the determination of species biomasses as well as abundances (simple blotted
720 wet-weights would suffice). This would open more opportunities for the assessment of
721 anthropogenic disturbance, for example the abundance / biomass comparison (ABC)
722 method or the phylum level meta-analysis (see Clarke & Warwick 2001). In the ABC
723 method, separate *k*-dominance curves for species abundance and species biomass act as
724 internal controls against each other, providing a snapshot of ecological condition that
725 obviates the need for reference samples in space or time (Warwick 1986; Warwick et al.
726 1987; Warwick and Clarke 1994). The phylum level meta-analysis compares the
727 proportional ‘production’ of higher taxa (based on a combination of abundance and
728 biomass) at a location with a training data set comprising a range of pollution/disturbance
729 scenarios (Warwick and Clarke 1993; Savage et al. 2001; Somerfield et al. 2006).

730 The UK’s approach to setting a conservation objective for a marine SAC feature includes
731 as an attribute “range of biotopes” and as a target “number of biotopes should not deviate
732 from baseline” (Figure 1-1 in Davies et al 2001). In view of the difficulty in ascribing the
733 assemblages on St Martin’s Flats to recognised biotopes in the JNCC or EUNIS level 5
734 classifications, this seems to be an impractical aspect of the approach. We would
735 recommend a more robust approach to defining species assemblage composition, tailored
736 to specific sites (as we have done above for St Martin’s Flats), rather than forcing these
737 assemblages to conform with previously recognised biotopes, or creating new ones. Such
738 habitat classifications are obviously acceptable up to level 3 (habitat complexes such as
739 littoral sand) that utilise only physical characters, but not at level 4 and above where
740 faunistic composition become part of the habitat definition.

741 Davies (1990) described the area as follows: “St Martin’s Flats is the largest continuous
742 area of sand in the Isles of Scilly. Tidal currents vary over the area resulting in different
743 degrees of sediment sorting which in turn leads to different infaunal communities. Small
744 populations of amphioxus, *Branchiostoma lanceolatum* are occasionally present. Large
745 populations of the sand mason *Lanice conchilega* extend from mid to low tide level. Rich

746 infaunal communities were characterised by heart urchins and bivalve molluscs,
747 including the uncommon species *Lutraria lutraria*. Nichols and Harris (1982)
748 recommend that these sediment shores be considered for statutory protection in view of
749 their high habitat diversity and associated species richness.” Statutory protection was put
750 in place, and as a result the Flats have been regularly monitored, showing that Davies’
751 description is as good now as it was then. The question that needs to be considered, then,
752 is whether the use of a biotope classification has helped in any way in this process or,
753 indeed, has it hindered?

754

755

756 **4. Acknowledgements**

757

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