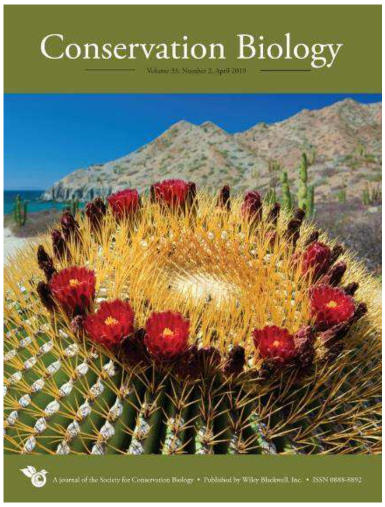

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Monitoring mosaic biotopes in a marine conservation zone by autonomous underwater vehicle

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Abstract

The extent of marine protected areas (MPAs) has increased dramatically in the last decade, and poses a major logistic challenge for conservation practitioners in terms of spatial extent and the multiplicity of habitats and biotopes that now require assessment. Here we demonstrate a single field method, photographic assessment by autonomous underwater vehicle (AUV) that enables the consistent description of multiple habitats, in our case including mosaics of rock and sediment. We describe a case study in the Greater Haig Fras marine conservation zone (Celtic Sea, NE Atlantic) where we distinguished seven biotopes, detected statistically significant variations in standing stocks, species density, species diversity, and faunal composition, and identified significant indicator species for each habitat. Our results demonstrate that AUV-based photography can produce robust data for ecological research and practical marine conservation. We note that standardizing to a minimum number of individuals per sampling unit, rather than to a fixed seafloor area, may be a valuable means of defining an ecologically appropriate sampling unit. Although representing a change in 'standard practise', we suggest that other users consider the potential benefits of this approach in their own conservation studies. The approach is broadly applicable in the marine environment, and has already been successfully implemented in deep-sea conservation and impact studies. It is clear that without a cost-effective methodology, applicable across habitats, it will be difficult to progress a coherent classification of biotopes, or to routinely assess their conservation status, in the rapidly expanding global extent of MPAs.

Keywords: marine protected area, seafloor, benthos, biotope classification, mosaic habitats, ecological metrics

Introduction

Acquiring ecological data is key to basic biological research, monitoring change in biodiversity, and the development of effective conservation actions. Achieving those aims in a timely and cost-effective manner remains a significant challenge in terrestrial and aquatic systems. In both cases, drones – unmanned aerial vehicles (UAVs) and autonomous underwater vehicles (AUVs) – offer the promise of significant advances in capability (Anderson & Gaston 2013; Wynn et al. 2014).

Marine protected areas (MPAs) have long been suggested as a tool for maintaining and restoring biodiversity (Woodcock et al. 2017), and the designation of very numerous MPAs is now driving the need for better, and more cost-effective, description and quantification of the habitats and biological assemblages present. AUVs are an established technology in seafloor research (Durden et al. 2016c), and appear to be an effective tool in science-driven and conservation-driven studies in shelf-sea (Marzinelli et al. 2015) and deep-sea studies (Morris et al. 2016). They offer rapid, non-destructive, data collection, access to a wide range of habitats, and reduced survey costs (Wynn et al. 2014). AUV data can improve the quantification of conservation metrics (Durden et al. 2016a), and may be of particular value in habitats where remote sampling methods are ineffective, such as reef or rock habitats (Tolimieri et al. 2008).

MPAs typically encompass multiple habitats, and the use of varying samplers (e.g. grabs, trawls, towed-cameras) has limited the degree to which the resultant data can be synthesised across habitats. The European Nature Information System (EUNIS) provides a classification of habitats and biotopes that has been influential in standardising habitat description (Costello 2009), although its limitations have become evident as conservation-based marine mapping has expanded. In particular Galparsoro et al. (2012) note that important mixed, or mosaic, marine habitats “cannot be represented using the current EUNIS classification system as it only recognises separate rock or sediment habitats”. Mosaic habitats likely play a key role in the connectivity that underpins the functioning of MPA networks (Olds et al. 2016), and how they might best be classified remains an area of active debate (Dauvin 2015). It is the rule-based hierarchical nature of EUNIS (e.g. rock or sediment) that poses the problem, which may similarly impact other hierarchical systems (Harris 2012).

Where habitat-type-dependant field methods are employed, a single biotope classification scheme can be difficult or impossible to operate (Van Rein et al. 2009). Variant field methodologies also introduce major mismatches in both the spatial scale observed and in the corresponding body sizes and taxonomic groups assessed. These difficulties could be reduced, and the full potential of AUV-based monitoring realised, if visual assessment by photography can be implemented usefully across multiple biotopes. The benefits would include: (i) common scales and methodology across habitats, and consequently a common classification scheme; (ii) explicit recording of the species and habitats that underpin MPA designation and legislation; (iii) direct evidence of violating activities. However, as Galparsoro et al. (2012) indicate, two questions remain: 1. How robust are visually-based classifications? and 2. What constitutes an appropriate sampling unit in photographic assessments?

To tackle these questions we undertook an AUV survey in the Greater Haig Fras marine conservation zone (Fig. 1a, b; Wynn et al. 2014). Nested within the MCZ is the Haig Fras special area of conservation (SAC) that includes a bedrock outcrop reef. The MCZ has substantial areas of mixed rock-sediment habitat that are difficult to assess by physical sampling. We use AUV data to: (i) investigate whether mosaic biotopes can be adequately described and discriminated on the basis of visual data; (ii) establish potential links between biotope characteristics and substratum type and complexity, to demonstrate the potential effectiveness of the method; and (iii) examine the influence of sampling unit choice in a practical conservation assessment of complex habitats.

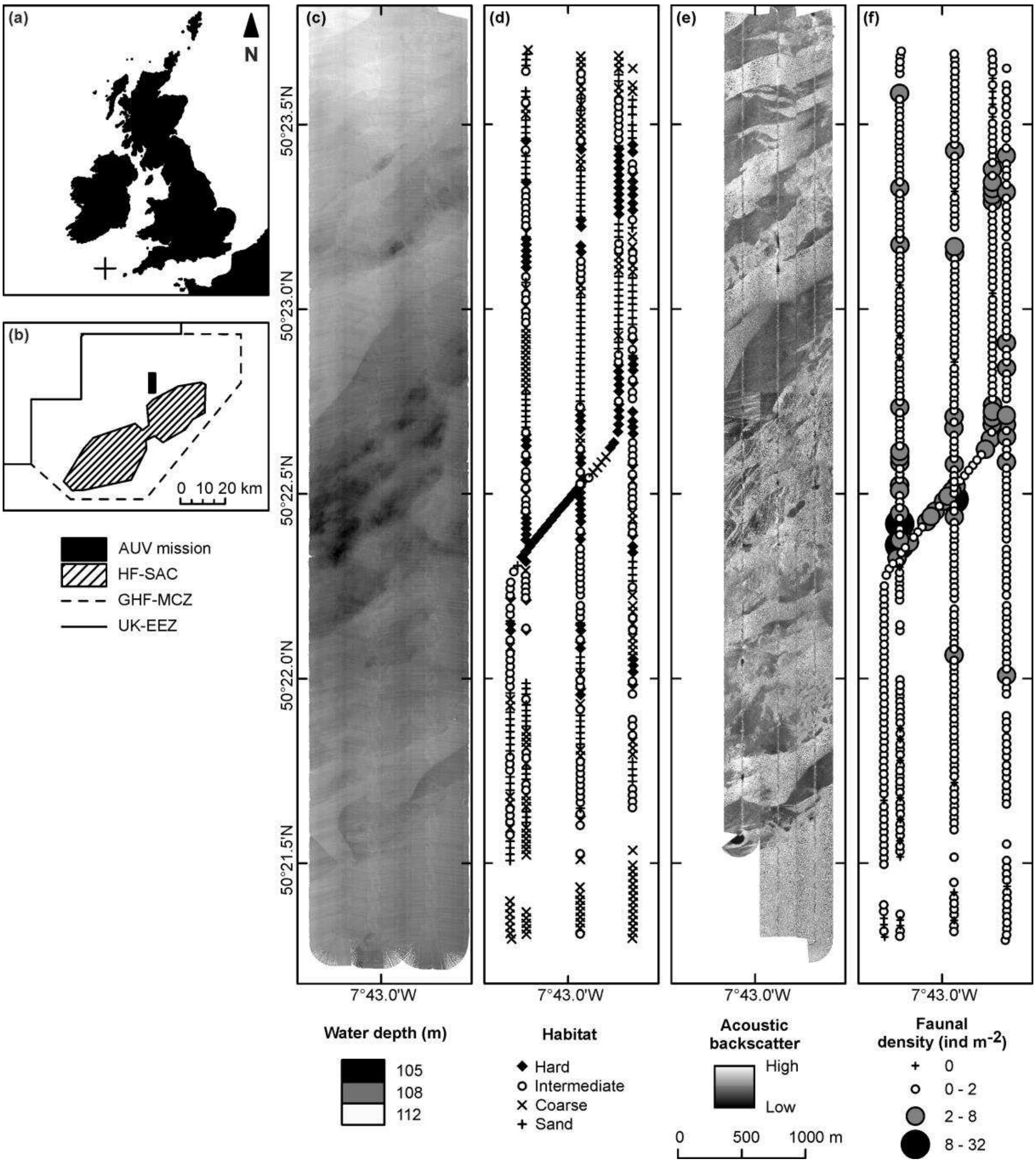

Fig. 1. Survey location. (a) Greater Haig Fras marine conservation zone in the Celtic Sea (GHF-MCZ). (b) Area of AUV survey and adjacent Haig Fras special area of conservation (SAC) within the GHF-MCZ. (c) Bathymetry. (d) Photographic habitat classification. (e) Sidescan sonar backscatter. (f) Faunal density.

Methods

Field survey

All data were derived from one 16-hour deployment of the AUV *Autosub6000* (July 2012; Ruhl 2013) during which the vehicle undertook three dives. Dive 1: swath bathymetry survey (Fig. 1c). Dive 2: photographic survey from a target altitude of 3.2 m using a Point Grey Research Grasshopper 2 camera (Morris et al. 2014). Dive 3: sidescan sonar survey (Fig. 1e). The swath bathymetry and sidescan sonar surveys are detailed here for completeness; in the assessment that follows, all data were derived from the single photographic survey dive (duration 225 minutes). The photographic survey was carried out as four north-south transect lines and a crossing line (Fig. 1d, f) that targeted a rock outcrop of slightly elevated terrain (Fig. 1c) with sinuous striations in the sonar view (Fig. 1f).

Image data generation

Images were processed to improve non-uniform illumination and colour representation, rectified to a common scale (0.59 mm pixel^{-1}), georeferenced, and mosaicked into groups of five consecutive images (tiles; Morris et al. 2014); in total 2637 such tiles were produced (each c. 7.3 m^2 seabed). Tiling was undertaken to remove overlap from consecutive photographs, and as a practical convenience to reduce the data management overhead. Tiles were assessed in random order to avoid bias through knowledge of spatial proximity (Durden et al. 2016b). We present results from three distinct sampling units: (a) Tile, sampling unit = primary sampling element, physical scale c. 7.3 m^2, variable number of specimens; (b) Composite area, sampling unit = multiple tiles, c. 150 m^2, variable number of specimens; (c) Composite individuals, sampling unit = multiple tiles, c. 150 specimens, variable seabed area (Table 1).

Seabed assessment. Three primary substratum types were recorded: hard substrata (bedrock, boulder, cobbles), coarse sediment (gravelly sand, granules, pebbles, shells), and sand. A primary substratum type was attributed based on majority (≥50%) tile area, and a secondary type was recorded if present (≥10%). The combination of primary and secondary types yielded four mixed, or mosaic, substratum categories (e.g. Post et al. 2011; Supporting Information). For presentation and analysis, the substratum classes were also simplified into summary habitats (Table 1): Hard habitats with hard primary substratum, Intermediate habitats with hard secondary substratum, and Coarse habitats and Sand habitats (jointly referred to as Sedimentary habitats) where hard substratum was absent. Note that we did not observe the presence of a Coarse and Sand mosaic. Litter and other human debris on the seabed were also recorded (Supporting Information).

Faunal assessment. Invertebrate and demersal fish (>1 cm body length) were counted, measured, and identified to the lowest taxonomic or morphotype unit possible (see e.g. Althaus et al. 2015). For colonial and encrusting organisms, the greatest diameter of individual colonies, or patches, was measured. Solitary tubicolous polychaetes, bivalves, and gastropods were observed but excluded from the analyses to avoid inclusion of empty tubes or shells. Indeterminate specimens (<1% of total) were excluded from subsequent analyses. Body-size measurements were converted to estimated wet weight biomass via existing length-weight relationships (Supporting Information).

Table 1. Photographic effort by habitat and substratum type, given as total survey and composite sample values (Ind., individuals).

		Total				Composite area			Composite individuals		
Habitat	Substratum[a]	Tiles	Area (m^2)	Area (%)	Ind.	n	Area (m^2)[b]	Ind.[b]	n	Area (m^2)[b]	Ind.[b]
Hard	H	121	882	4.6	2832	6	147	472	19	16	149
Hard	Hc	211	1564	8.1	3648	10	156	265	59	27	147
Hard	Hs	214	1656	8.6	4135	10	165	414	61	27	148
Intermediate	Ch	584	4255	22.1	1476	29	146	51	12	355	148
Intermediate	Sh	119	874	4.5	389	6	145	65	12	73	130
Coarse	C	669	4836	25.2	446	33	146	14	3	1612	149
Sand	S	719	5156	26.8	966	36	143	27	6	859	138
Mean							150	187		229	147
Total		2637	19223	100.0	12892	130			84		

[a] Primary substratum (H)ard, (C)oarse, and (S)and, secondary substratum corresponding lower case.
[b] Mean of replicates.

Faunal community analysis

Statistical approach. We considered the complete set of tiles to represent the total population, i.e. assessments were carried out within that population, and we make no statistical inference beyond that population. Our primary objective was to test for biological differences between habitats; we therefore first grouped the tiles by substratum type. In our case, and in many marine settings, a single photograph (or tile) was insufficient to establish a useful estimate of species diversity or species composition, consequently we compiled the data from multiple tiles to form the sampling units (replicates) of this study. Given the non-independent nature of consecutive tiles and the inevitable occurrence of spatial autocorrelation (Legendre 1993), we compiled the data from individual tiles at random within substratum type to form composite area sampling units of c. 150 m^2 per replicate (Table 1). A simplified illustration of this methodology and formal testing of the randomisation process are included with the online version of this article (Supporting Information). We also wished to test the effect of sampling unit choice; this was done in the same manner (composite individuals sampling units of c. 150 individuals per replicate; Table 1; Supporting Information).

Density and biomass. Individual tile data were log-transformed and assessed using Welch's one-way ANOVA, with subsequent pairwise comparisons made using the Games-Howell method, as implemented in Minitab (V17, Minitab, Inc.). To estimate density and biomass at physical scales greater than a single tile, data were repeatedly, randomly, accumulated with replacement to form larger physical samples of 2 to 724 tiles, and a median value was derived from the repeats (in R environment; R Core Team 2016).

Diversity and composition. Faunal diversity was assessed with replicate-level data (Table 1) using sample-based rarefaction of taxon richness (Sest; Colwell et al. 2012), the exponential form of the Shannon Index (expH´; Magurran 2004), and the inverse form of Simpson's Index (1/D; Magurran 2004), as calculated via 1000 randomisations without replacement for Sest, and with replacement for expH' and 1/D, in EstimateS (V9.1.0, Colwell 2013). Faunal composition was assessed by non-metric multidimensional scaling ordination (nMDS) based on the Bray-Curtis dissimilarity of log-transformed faunal density data, and subsequent analysis of similarities (ANOSIM), all implemented using PRIMER (V6.1.11, Quest Research Ltd; Clarke & Warwick 1994). Morphotype specificity and fidelity to particular substratum types was assessed by the indicator value method, as implemented in the R package 'indicspecies' (De Cáceres & Legendre 2009), and by two-way indicator species analysis (TWINSPAN), as implemented in the software package PC-ORD (V4, Wild Blueberry Media LLC) using five logarithmically arranged density levels. To evaluate the choice of sampling unit we produced auto-similarity curves (Schneck & Melo 2010), as employed by Durden et al. (2016b) in an assessment of seabed photography. The method calculates the average Bray-Curtis dissimilarity between pairs of composite samples formed from increasing numbers of tiles by random resampling of the original data within habitat type (1000 times without replacement; in the R environment).

Results

Standing stocks

Whether assessed using tile-level or composite-area-replicate data, faunal density exhibited a statistically significant difference between habitats (Welch's ANOVA, $p < 0.001$; Fig. 2a, b), with Hard habitats having the highest density, and Coarse the lowest. All pairwise comparisons were significant (Games-Howell, $p < 0.05$). Area-scaled density by habitat followed the same trends, with apparent median density rapidly stabilising with seabed area assessed in all habitats (Fig. 2c). Faunal biomass also varied significantly between habitats when assessed using tile-level data (Welch's ANOVA, $p < 0.001$; Fig. 2a), with Hard habitats having the highest biomass and Coarse the lowest. Pairwise comparisons indicated

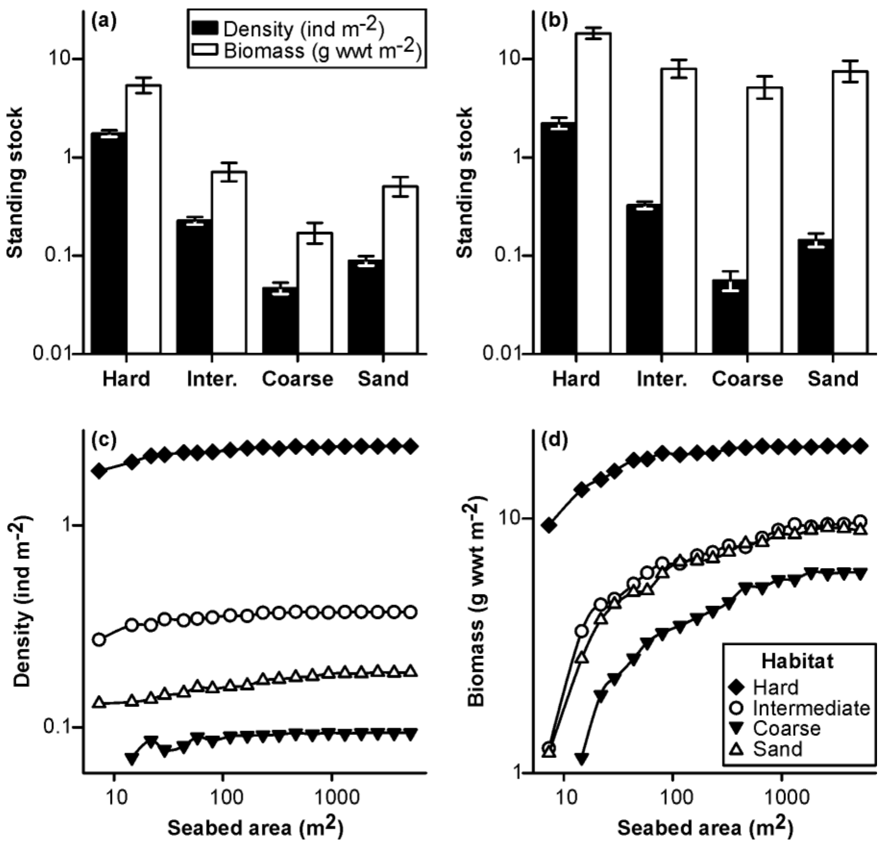

Fig. 2. Standing stock by habitat determined at (a) tile scale (c. 7.3 m^2) and (b) composite area sample scale (c. 150 m^2), illustrated as geometric mean and 95% confidence interval, together with (c) median density, and (d) median biomass estimated from increasingly large seabed areas.

significant differences between all habitats (Games-Howell, $p < 0.05$), except between Intermediate and Sand (Games-Howell, $p = 0.14$). When assessed using composite-area-replicate data, biomass also varied significantly between habitats (Welch's ANOVA, $p < 0.001$; Fig. 2b), however the magnitude of differences was substantially reduced. Pairwise comparisons indicated significant differences between all habitats (Games-Howell, $p < 0.05$), except between Intermediate and Sand (Games-Howell, $p = 0.98$), and Coarse and Sand (Games-Howell, $p = 0.15$). Area-scaled biomass by habitat followed the same trends, however, apparent median biomass was slow to stabilise with seabed area assessed, stabilizing at c. 650 m^2 in Hard habitats, and at c. 2000 m^2 in all other habitat types (Fig. 2d).

Faunal diversity

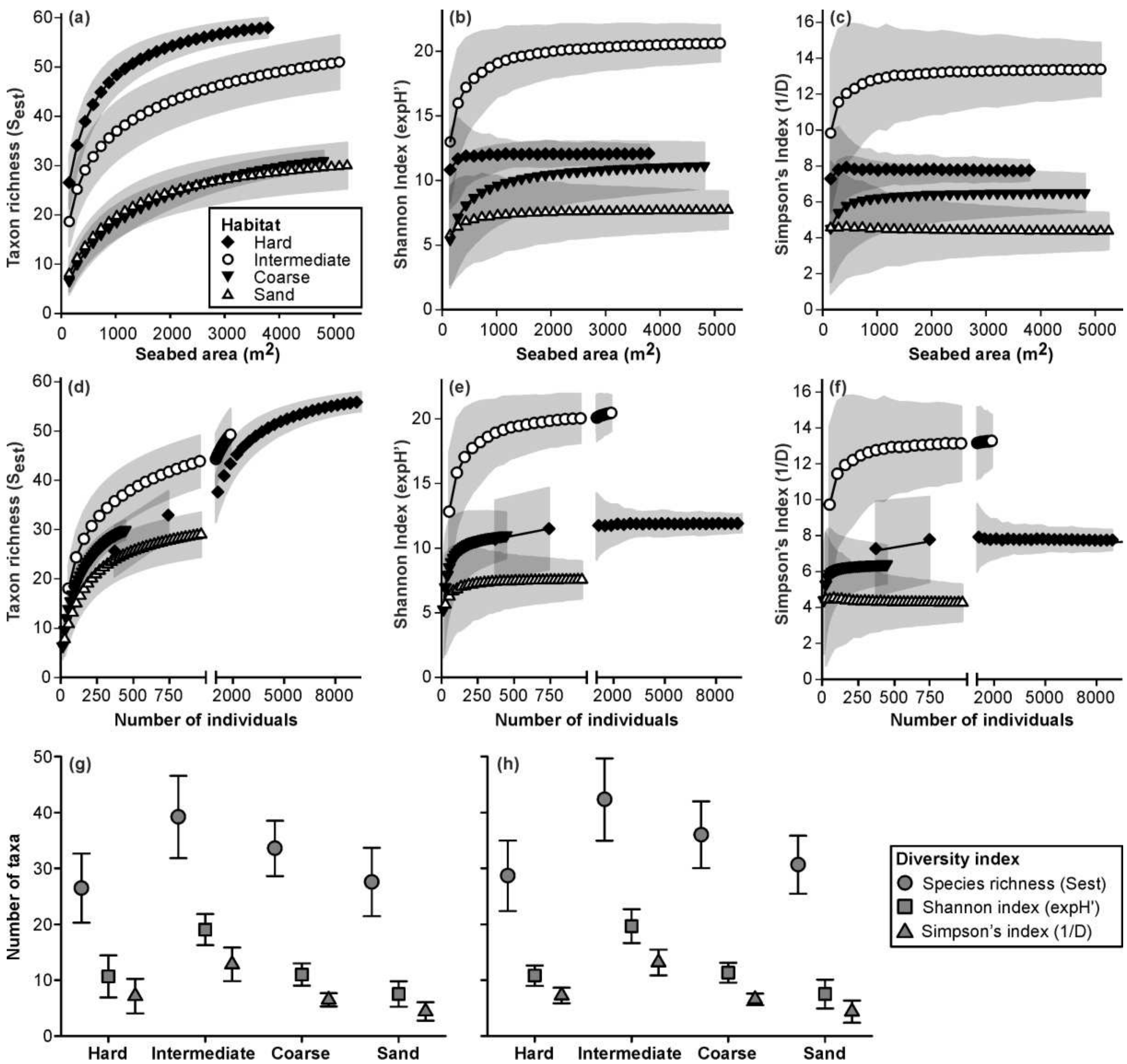

Fig. 3. Sample-based rarefaction of diversity by habitat for (a-g) composite area samples, and (h) composite individuals samples, as mean and 95% confidence interval (shaded area or error bar). (g) Simplified results for composite area samples, illustrated at an approximately equal number of individuals (364-375) across habitats. (h) Simplified results for composite individual samples, illustrated for the three sample case, having an approximately equal total number of individuals (446-483) across habitats.

Assessed by composite area replicates, taxon richness (Sest) exhibited statistically significant differences between habitats, with Hard and Intermediate being notably richer than both Coarse and Sand habitats (Fig. 3a). Note, however, that these differences were less clear-cut when rarefied by number of individuals (Fig. 3d). In contrast, heterogeneity diversity (expH') and dominance diversity (1/D) showed consistent, statistically significant differences between Intermediate and other habitats whether rarefied by area or individuals, with Intermediate the most diverse, and Sand the least (Fig. 3b, c, e, f). These patterns were consistent whether analysed on the basis of composite area or composite individuals replicates (Fig. 3g, h).

Faunal composition

Faunal composition in composite area replicates varied significantly with substratum type (ANOSIM, R = 0.80, $p < 0.001$). Ordination suggested three distinct sample groupings corresponding with the Hard, Intermediate, and Sedimentary habitats (Fig. 4a), that were ordered by the relative occurrence of hard substratum. Within each of these three primary groups, samples were also well ordered by the relative occurrence of coarse and sand substrata (Supporting Information). All pairwise comparisons of faunal composition by substratum type were statistically significant (ANOSIM R = 0.36 - 1.00, $p < 0.05$; Supporting Information). Indicator species analysis suggested numerous taxa as statistically significant indicators for Hard habitats, single taxa for the Intermediate and Coarse habitats, and three taxa for the Sand habitat (Table 2; Supporting Information). Two-way indicator species analysis (TWINSPAN) almost perfectly divided the samples into the visually determined summary habitat classes; all Hard (n = 26), Intermediate (35), and Coarse (33) samples were correctly classified, with four of the 36 Sand samples misclassified as Coarse (Table 2).

Faunal composition in composite individuals replicates also showed very clear groupings corresponding with the Hard, Intermediate, and Sedimentary habitats, and separation of the Coarse and Sand habitats (Fig. 4b). All pairwise comparisons of faunal composition between

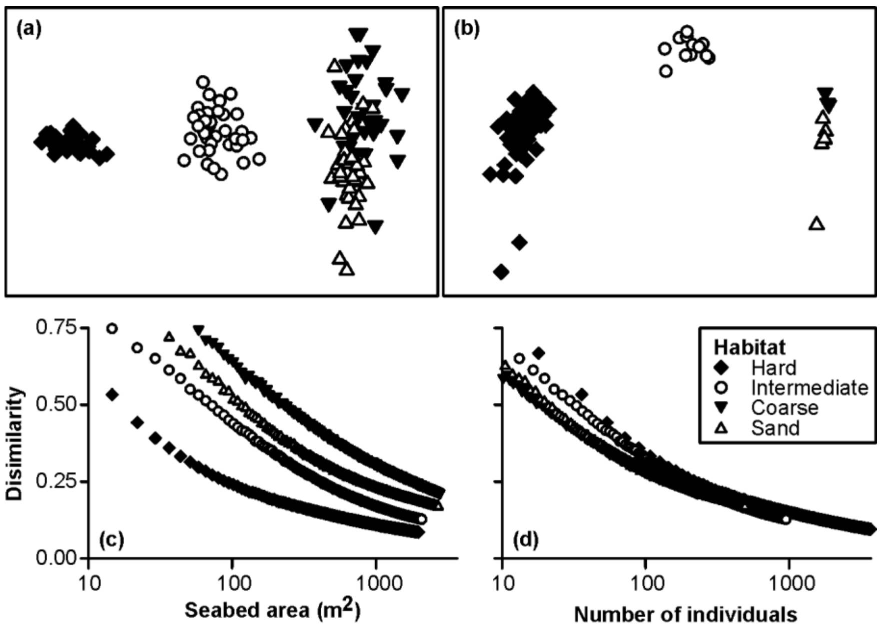

Fig. 4. Variation in faunal composition and auto-similarity with habitat type. (a) 2D non-metric multidimensional scaling ordination of Bray-Curtis dissimilarity of log-transformed density in composite area samples, and (b) composite individuals samples. Auto-similarity curves plotted by (c) seabed area sampled, and (d) number of individuals sampled.

Table 2. Summary of indicator species analyses, indicator species (bold) and preferentially occurring taxa are listed together with corresponding composite area sample groups, and frequency of occurrence in (H)ard, (I)ntermediate, (C)oarse, and (S)and habitats.

Two-way indicator species analysis					Indicator species analysis				
D1[a]	Taxa	D2[a]	Taxa	Samples[b]	Taxa[c]	Frequency (%) H	I	C	S
1	**Bryozoa 01** Porifera 23 Axinellidae spp.	1.1	**Axinellidae spp.** *Porella* sp. *Parazoanthus* sp.	26 × H	***Parazoanthus* sp.**	100.0	34.3	3.0	0.0
					Axinellidae spp.	100.0	77.1	0.0	0.0
					***Porella* sp.**	100.0	74.3	0.0	0.0
					Porifera 20	100.0	28.6	0.0	0.0
					Salmacina dysteri	100.0	65.7	0.0	5.6
					***Munida* sp.**	100.0	74.3	3.0	5.6
					Echinus esculentus	96.2	37.1	0.0	0.0
					***Reteporella* spp.**	100.0	40.0	0.0	5.6
					Stichastrella rosea	100.0	60.0	27.3	19.4
					***Antedon* spp.**	80.8	28.6	3.0	2.8
		1.2	Anthozoa 34 Anthozoa 39 *Lepidorhombus whiffiagonis*	35 × I	**Anthozoa 39**	7.7	42.9	12.1	2.8
2	Perciforme spp. 10 Gadidae spp. Paguridae 02	2.1	***Bolocera* spp.** Anthozoa 16	33 × C 4 × S	**Paguridae 01**	3.8	0.0	21.2	8.3
		2.2	**Paguridae 02** **Cerianthid 01** **Anthozoa 03**	32 × S	**Perciforme spp. 10**	0.0	5.7	6.1	33.3
					***Liocarcinus* spp.**	0.0	5.7	3.0	25.0
					Hippoglossoides platessoides	3.8	2.9	0.0	19.4

[a] First (1) and second (2) hierarchical divisions of samples.
[b] Number of samples from each habitat classified in corresponding division.
[c] Statistically significant (p < 0.05) indicator species, note only top 10 of 28 are listed for Hard habitats.

habitats were statistically significant, with strong differentiation in most comparisons (ANOSIM R = 1.0, $p \leq 0.002$), except between Coarse and Sand, which were nonetheless statistically significant (ANOSIM R = 0.53, p = 0.036). Auto-similarity curves for the four summary habitats showed considerable variation when assessed in terms of seabed area sampled (Fig. 4c). That variability was substantially reduced when assessed in terms of the number of individuals sampled (Fig. 4d), reflecting the major difference in faunal density between habitats (e.g. Fig. 2). To achieve a target assemblage description level of 0.75 self-similarity, composite area samples would vary from 90 to 1840 m^2 between habitats, or only from 140 to 220 specimens per composite individuals sample.

Discussion

The area surveyed was characterised by the presence of sand and coarser-grained sedimentary environments, together with outcropping bedrock, boulder, and cobble substrata. We believe that the variety and complexity of the physical environment of the Greater Haig Fras marine conservation zone represents a good testbed for the conservation assessment of other large marine protected areas. From an ecological perspective: the presence of hard substrata exerted a strong positive control on faunal density, biomass, and total species richness; mosaic habitats substantially enhanced faunal diversity; and all habitats and mosaics supported distinct faunal assemblages. Photographic assessment provided a uniform field- and data-analysis methodology across rocky and sedimentary habitats that enabled us to make a direct assessment of multiple biotopes and their occurrence in mosaic form. This ability to resolve ecologically significant information, at broad-scale, across multiple and mixed habitats, suggests that the AUV-based photographic survey was an effective and efficient practical conservation management tool in the present case, and indicated its potential value in other similarly complex marine habitats.

Mosaic habitats

Intermediate habitats, or mosaics of hard substratum type within a sedimentary matrix, represent one-quarter of the seafloor area observed. Their ecological characteristics were largely predictable as an admixture of their component habitats, and consistent with a simple ecotone concept (Odum & Barrett 2005). Faunal density in Intermediate habitats was significantly different from, and transitional to, both Hard and Sedimentary habitats. Regardless of whether rarefied by individuals or seabed area, heterogeneity diversity measures were significantly elevated in Intermediate habitats over both Hard and Sedimentary habitats. This suggests that the addition of the two assemblages (i.e. Hard and Sedimentary) acted to reduce the dominance component of diversity in the combined assemblage.

When taxon richness was assessed as species density (Whittaker et al. 2001), Intermediate habitats were significantly different from, and transitional to, both Hard and Sedimentary habitats (Hard > Intermediate > Coarse ~ Sand). However, when assessed as number of species per individual, the habitats were not statistically distinct and were ordered differently (Intermediate > Coarse > Hard > Sand). Species density and total faunal density exhibited the same pattern, and might both be controlled by resource availability. In contrast, heterogeneity diversity appeared to exhibit a different pattern related to seafloor habitat complexity: uniform sediment (Sand) < mixed sediment (Coarse) < topographically complex cobble / boulder / bedrock (Hard) < mosaicked hard substratum 'islands' in a sedimentary matrix (Intermediate). Environmental heterogeneity is thought to be a key driver of species richness (Yang et al. 2015), as was evident in the present study, although the effect was more pronounced in the case of heterogeneity diversity.

Mosaic habitats are thought to play a key role in the connectivity of marine ecosystems, in terms of both secondary productivity and the maintenance of biological diversity (Olds et al. 2016). They can represent corridors, or stepping-stones, facilitating the movement of organisms, and thereby processes, between dispersed primary habitats. In the case of Haig Fras, the SAC protects what is thought to be the only substantial area of offshore rocky reef habitat in the Celtic Sea. The substantial presence of mosaic habitats in our survey area, and more widely in the Celtic Sea (Thompson et al. 2017), indicates both the potential connectivity of dispersed rocky reefs in the region and the need to protect some of that mosaic habitat in the background environment. These observations provide strong support for the calls to both record (classify) and quantify these mosaic habitats (e.g. Galparsoro et al. 2012; Dauvin 2015). There is also an obvious need to define the physical scales at which the occurrence of mosaics are practically assessed and at which conservation policies might be applied. The quality of the intervening matrix environment may determine the effectiveness of connectivity (Baum et al. 2004), and has been a matter of concern in terrestrial conservation schemes (Donald & Evans 2006).

Practical conservation

The UK has implemented over 200 MPAs, with over 27 million km^2 of MPA now designated globally (UNEP-WCMC and IUCN 2019). The routine monitoring of such a large network implies substantial financial costs. We consider that AUV-based assessment offers a cost-effective solution (Wynn et al. 2014). Our survey can be approximated as a 20-km track accomplished at 1.38 ms^{-1} (2.7 knots), i.e. c. four-hour duration. Fitting an identical camera and image storage system to a towed platform, or remotely operated vehicle, and operating at 0.26 ms^{-1} (0.5 knots), the survey would require at least 21 hours of ship time. In the case of a towed platform, sea state (swell waves) can be expected to render c. 25% of images unusable, and therefore the effective survey speed 0.20 ms^{-1}, the full survey then requiring at least 28 hours of ship time. Consequently, in the case of our survey, the AUV-based approach offers a potential 86% saving on ship-time cost / carbon footprint compared to an equivalent towed camera survey, and perhaps a 96% saving if the ship carries out other useful work for three hours while the AUV is submerged.

In terms of cost-effectiveness and conservation-effectiveness, survey design may be a key factor, raising two fundamental questions: what sampling unit is required to obtain suitably accurate and precise data (Galparsoro et al. 2012), and how should the survey be conducted (Foster et al. 2014). Our study demonstrates that AUV photography can provide enhanced information on the nature of the substratum and its associated fauna. The distribution of the identified habitat types closely matched the sidescan sonar mapping, suggesting consistency and accuracy in the visual assessment method. That we have been able to detect statistically significant differences in the key ecological parameters (standing stock, species richness and diversity, faunal composition, and indicator taxa) suggests that the technique can produce suitably robust data. Visual monitoring also provided direct evidence of human impacts in the form of lost / discarded fishing gear and plastic debris at the seabed (Supporting Information).

Although our survey was undertaken in a fixed grid form, suited to the complete bathymetric and sidescan sonar mapping of the area, it is important to note that our subsequent treatment of the photographic data changed the character of the biological survey. By partitioning the seafloor into substratum types, and then randomly forming sampling units within those types, we converted the non-random grid survey to a form of a posteriori stratified random sampling scheme. It is also important to note that in our study we were able to identify seafloor habitat type at a much smaller physical scale (1 m^2) than we think is necessary to appropriately sample the associated fauna ($\geq$150 m^2). This point may be particularly important in the development of cost-effective monitoring for complex marine habitats.

There are many potential options for AUV survey design (Foster et al. 2014), however, their implementation may require prior knowledge of environmental stratification and / or the appropriate sampling unit. Consequently, the combined a posteriori stratification and composite sampling that we have adopted here may have broad, cost-effective, general application in many marine systems, perhaps particularly in spatially complex environments (Huvenne et al. 2011; Thornton et al. 2016). Our approach is potentially applicable to any image dataset that can be partitioned into ecologically relevant subsets based upon some known or identifiable environmental variable(s). For example, (i) Morris et al. (2016) segregated their data by topographic height to contrast the ecology of a small abyssal hill with that of the surrounding plain (NE Atlantic); (ii) Simon-Lledó et al. (2019c) assessed ecological variation over a manganese nodule occurrence gradient in the Clarion-Clipperton Zone (NE Pacific), partitioning their data by seafloor nodule coverage using an automated detection technique (Schoening et al. 2017); (iii) Simon-Lledó et al. (2019b) assessed the long-term impact of simulated deep-sea mining in the Peru Basin (SE Pacific) by segregating their data on proximity to 26-year old seabed plough marks.

Our results suggest that parameters of conservation value exhibit various responses to the choice of sampling unit, primarily linked to the number of specimens encompassed: (a) Numerical density (Fig. 1c) is essentially insensitive to unit size; (b) Biomass density (Fig. 1d) is highly sensitive to unit size, linked to the power law distribution of individual body sizes (Bett in press); (c) Species richness (Fig. 3a,d) has a long established link to sampling unit size (Sanders 1968; Colwell et al. 2012); (e) Faunal composition (similarly; Fig. 4) is also substantially influenced by unit size. In the case of biomass and species richness, unit size has a direct impact on the value (accuracy) of the measured parameter. In the case of faunal composition, unit size impacts on the variability (precision) of resulting assessments, i.e. the ability to define, discriminate, or monitor the status of a given assemblage / biotope. Similar conclusions were reached by Simon-Lledó et al. (2019a) in their assessment of the effect of sampling unit size in describing assemblages based on AUV photography.

Anderson and Santana-Garcon (2015) tackle the issue of variability in faunal composition in a similar manner to the present study, by pooling subsamples and asking how many original smaller-scale sampling units are needed to provide a reasonable measure of community structure for comparative analysis. Defining what is 'reasonable' is likely to require case-by-case consideration of specific survey objectives. Forcino et al. (2015) have considered the appropriate minimum number of specimens per sampling unit across a broad range of terrestrial and aquatic community types. They suggest a minimum number of 58 individuals per unit to be adequate for multivariate analyses. However, they note that this number is likely to be higher in situations where (a) assemblage evenness is high, (b) assemblage taxon richness is high, and (c) where ecological contrasts (in space or time) are low.

In the present study, we based our assessment of the appropriate number of individuals per sampling unit on a target within-habitat dissimilarity between replicates of 0.25, yielding a range of c. 150-250 individuals per composite sample across habitats. We aimed at standardizing sampling effort between habitat-specific samples by equalizing dissimilarity between samples within the habitats of interest, rather than simply standardizing by seabed area examined. At a more complex level, an optimised data analysis strategy could potentially implement habitat-based rules, in our case: Sand ≥150 and Hard ≥250 individuals per composite sample.

Whether based on the auto-similarity curve approach we have adopted, or the assessment of multivariate dissimilarity-based standard error developed by Anderson and Santana-Garcon (2015), we suggest that users consider the potential value of defining their sampling units in terms of number of individuals rather than automatically adopting an area-defined unit. We suspect this approach may have broad application in marine conservation studies, particularly those based on photographic assessments, and should be simple to implement for mass photography from both ROVs and AUVs. We recognise that this may represent a substantial departure from 'standard practise', nevertheless, we suggest that users should consider the potential benefits to their own conservation status assessment and monitoring objectives. It is perhaps worth restating that the need to control the number of individuals examined in producing reliable comparative assessments of marine benthic diversity is a very long-established practise (Sanders 1968) and could valuably be expanded to both biomass density and faunal composition assessments.

Marine conservation capability is increasing rapidly with the availability of new technology. Methods for the automated classification of seafloor images are being developed in the quantification of phytodetritus cover (Morris et al. 2016), the characterisation of manganese nodule fields (Schoening et al. 2017), the identification and coverage estimation of kelp forests (Marzinelli et al. 2015), corals and macroalgae (Monk et al. 2018). However, the routine wide-spread use of automated detection and recognition of individual seafloor species occurrences is not yet possible; though progress seems certain in the coming years. We consider that AUVs are a mature technology, with several commercial systems available for photographic and acoustic mapping work, offering a practical step change in marine conservation capability. The use of mass photography to achieve such aims will, however, require some change in common practices. Given the goals of cost savings per survey, and a common methodology across biotopes and habitats, such change may be a key part of achieving a practical means to more effectively monitor the world's growing network of MPAs.

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Supporting Information

Seabed type classification (Appendix S1), litter and biological features (Appendix S2), length-weight relationships (Appendix S3), composite sample formation (Appendix S4), testing of randomisation process (Appendix S5), multivariate analyses of composite area samples (Appendix S6), and indicator species (Appendix S7) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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