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Development of low-cost image mosaics of hard-bottom sessile communities using SCUBA: comparisons of optical media and of proxy measures of community structure

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Underwater image-based sampling procedures, using SCUBA, are compared using imagery collected from a temperate hard-substratum community. The effectiveness of a low-budget, high-resolution image mosaicing technique is assessed by comparing the relative efficiency of data collection, extraction and analysis among sampling procedures. In addition, a manipulative experiment tested whether the sampling procedures could detect the physical removal of 10% of the reef community. Overall, four factors were explored within the data: data collection media (stills and video), cover and community composition estimation techniques (visual cover estimation, frequency of occurrence and point extraction), change detection (pre- and post-impact) and depth (8, 14, 18 and 22 m). Stills imagery sampled the reef community at a higher image resolution than the video imagery, which enabled identification of more species and less-conspicuous benthic categories. Using the visual cover estimation technique, the stills imagery also had the greatest benefit in terms of efficiency and species identification. However, the experimental impact was detected using only the point extraction technique. The recommendations are that: (1) the image mosaicing technique is applied to fixed-station monitoring; (2) the point extraction technique be used for efficient and cost-effective monitoring at coarse taxonomic resolutions; and (3) survey depths remain constant over the duration of hard-substratum community monitoring.

Keywords: monitoring methods, image mosaics, cover estimation, community structure, subtidal rocky habitats, underwater cameras, temperate water

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INTRODUCTION

Shallow marine communities on hard substrata are often diverse and spatially complex in nature (Wood, 1999). Their study usually requires the use of SCUBA, rather than vessel-deployed sampling gear such as sediment grabs and cores (Davies *et al.*, 2001; van Rein *et al.*, 2009). With divers sampling these communities *in situ*, emphasis must be placed on safety, which favours study methods that reduce task-loading and the time spent underwater. Nonetheless, these methods need to be efficient, i.e. cost-effective and must also provide statistically robust data. In temperate waters, where tides, currents, water temperature and poor weather may often act against the researcher, this is particularly relevant (Shears, 2007).

Study methods using digital imagery can greatly reduce the time spent underwater, provide a permanent sampling record and be procedurally easy to follow, therefore reducing the need for skilled scientific divers for survey work. They are also considerably less invasive than most other methods when sampling more sensitive benthic habitats and therefore useful when monitoring within areas of conservation importance (Bohnsack, 1979). As a result, there is increasing use of underwater cameras in marine temperate hard-substratum studies, where either video- (Breen *et al.*, 2006; Barrett *et al.*, 2007; Goodwin *et al.*, 2009) or stills-based (Bell *et al.*, 2006; Burton *et al.*, 2007; Lock *et al.*, 2009) digital imagery are used to collect biological community samples.

The mosaicing of images collected from digital optical imagery can further improve the efficiency of survey work, and be of particular advantage in temperate waters where water-column visibility is an issue (Martin & Martin, 2002; Sayer & Poonian, 2007). The potential in mosaicing optical imagery lies with being able to create larger images of the seabed while maintaining high image resolution. Benthic community data can subsequently be collected from these images. Studies in deep water, well beyond safe SCUBA diving limits, i.e. >40 m depth, have focused on automatic mosaic generation, image classification and the georeferencing of mosaics (Gracias & Santos-Victor, 1998; Vincent *et al.*, 2003; Rzhanov & Mayer, 2004; Rzhanov *et al.*, 2006; Jerosch *et al.*, 2007). Of the mosaicing studies conducted within SCUBA diving limits (Gleason *et al.*, 2007, 2010; Lirman

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et al., 2007, 2010), little emphasis has been placed on developing mosaicing methods that are inexpensive and require less complex software and less skilled technicians to operate (Whittington *et al.*, 2006).

Method comparison studies appear to be the best approach to developing study methods that enhance cost-effectiveness and statistical robustness of surveys. Comparative studies have focused on appropriate technologies (Aronson *et al.*, 1994; Lam *et al.*, 2006; Leujak & Ormond, 2007; Dumas *et al.*, 2009), the dimensions of sampling units (Bowden, 2005; Houk & Van Woesik, 2006; Sayer & Poonian, 2007), levels of replication (Brown *et al.*, 2004; Jokiel *et al.*, 2005), effects of inter-observer variability (Benedetti-Cecchi *et al.*, 1996; Ninio *et al.*, 2003), data extraction techniques (Drummond & Connell, 2005; Beaumont *et al.*, 2007) and approaches to data analysis (Moore & Gilliland, 2000). Despite this wealth of knowledge, few standardized monitoring methods are in regular use in the hard-substratum environments of temperate waters, largely due to differences in survey environments, target communities and operational budgets (Davies *et al.*, 2001; Whittington *et al.*, 2006; Lock *et al.*, 2009).

With the advent of the Habitats Directive (EEC, 1992), the Water Framework Directive (EC, 2000) and the Marine Strategy Framework Directive (EC, 2008), a need has arisen for EU member states to develop methods for monitoring programmes, from which well-informed decisions regarding the status and appropriate management of marine environments could be made (Puente & Juanes, 2008). Indeed, this need is global (Diaz *et al.*, 2004). In some habitats, such as those with a soft substratum, long-established methods were merely adapted to meet the requirements of the directives (Borja *et al.*, 2000; Davies *et al.*, 2001; Rosenberg *et al.*, 2004). However, for the communities of hard substratum habitats, this has presented a greater challenge due to the structural complexity of resident benthic communities (Leonard & Clark, 1993) and the inherent problems of *in situ* sampling techniques (Shears, 2007). This has required the development of new and innovative methods (Whittington *et al.*, 2006; Lock *et al.*, 2009) to potentially parallel those in use in corresponding tropical habitats, where survey conditions are more amenable (Hill & Wilkinson, 2004; Jokiel *et al.*, 2005). In recognizing these needs, we developed a novel, low-cost image mosaicing method to collect high-resolution imagery from potentially turbid waters. The goals of this study are:

1. to assess the mosaicing method by comparing community data collected from the corresponding stills and video imagery;
2. to determine which of three data extraction techniques collects community data most efficiently and with most benefit from the mosaics;
3. to conduct a manipulative experiment to assess the sensitivity of the data extraction techniques to detecting a 10% change within the sampled communities; and
4. to assess differences in the community with changes in depth, using both stills and video imagery.

MATERIALS AND METHODS

Study site

This study was conducted off Black Head, located on the south-west shore of Rathlin Island, Northern Ireland (55°17.463N 6°15.150W). This embayment is within the Rathlin Island Special Area of Conservation (SAC), designated in part for the good conservation status of its rocky reef habitat, which boasts a rich community of taxa (Breen *et al.*, 2006). Despite having a narrow tidal range, with a value of 1.0 m at mean spring tides (Atkins, 1997), the sea around Rathlin Island experiences strong tidal forces, which due to variable bathymetry produce strong current eddies around the island's perimeter. However, water-column visibility is relatively clear due to low levels of suspended matter and is therefore suited to the testing of optical-based sampling methods. Four depth bands were chosen to be representative of the local infralittoral zone: 5–10 m, 10–15 m, 15–20 m and 20–25 m below mean sea level.

Quadrat collection

Stills and video imagery were collected sequentially during the same SCUBA dive from paired sets of 100 × 100 cm sampling quadrat frames, over a continuous two-week period in July 2008. We used a Nikon digital single-lens reflex (DSLR) camera in an Ikelite underwater housing (see Figure 1 for specifications), with purpose-built 25 × 25 cm photo-quadrat frame extending 40 cm outwards from the lens, to collect the stills 'image tiles' which we later used to construct the photo-mosaics. The zoom lens was set to a focal length of 25 mm. The dimensions of this photo-quadrat frame were based on the initial recommendations of Leujak & Ormond (2007), but adapted to suit the DSLR and housing used in this study. The 100 × 100 cm sampling quadrat frame was purposely divided into 16 equal areas, each measuring 25 × 25 cm, thus matching the dimensions of the photo-quadrat frame of the underwater housing. Once the sampling quadrat was held securely in place with weights, individual image tiles were collected from each of the 16 equal 25 × 25 cm areas by a SCUBA diver. It would have been impractical to move aside portions of the canopy biota to better sample the understorey biota (Dethier *et al.*, 1993). In the interests of improving efficiency and diver safety, the sampled communities were, therefore, treated as two-dimensional when data were extracted (Meese & Tomich, 1992).

A Sony digital handycam video camera in a Gates Sony underwater housing was used to collect comparative imagery from the same 100 × 100 cm sampling quadrat. With no corresponding frame built for this underwater housing, the SCUBA diver held the camera approximately 40 cm above the substratum under the sampling quadrat and progressed freely around the 16 individual 25 × 25 cm areas in the way as described above, but paused for at least 3 seconds over each area so that a clear still image could subsequently be extracted from the imagery for the video-mosaicing process. In this way, stills and video imagery from four replicate 100 × 100 cm sampling quadrats were collected from each of the four depth bands (8 m, 14 m, 18 m and 22 m, all within ± 1 m of the targeted depth). Data extracted from these sixteen quadrats constitute the pre-impact treatment of this study, and were taken to represent the natural, undisturbed state of the infralittoral community of Black Head.

Subsequently, a manipulative disturbance experiment was undertaken. Ten random 10-×-10-cm squares were placed within the area of the 100 × 100 cm sampling quadrat. Foliose macroalgae (>1 cm height above the substratum)

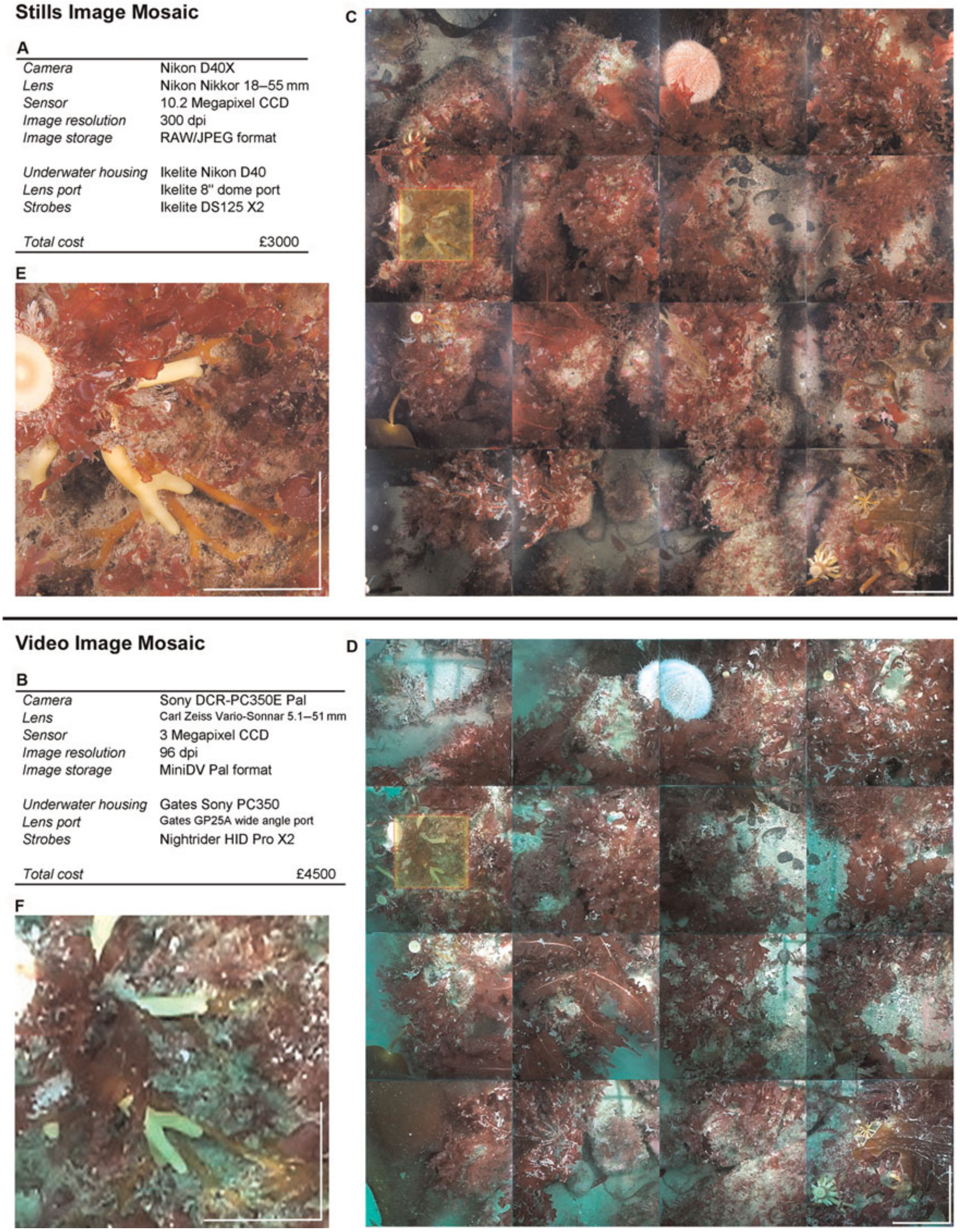

Fig. 1. Example stills and video image mosaics from the same sampling quadrat at 14 m depth. Technical comparisons and cost of field equipment to collect (A) stills and (B) video imagery. Full 100 × 100 cm (C) stills and (D) video image mosaics (inserts indicate areas from which (E) stills and (F) video enlargements demonstrate differences in image resolution). Note 10 cm and 5 cm white scale bars in bottom right corners of (C) and (D), and (E) and (F), respectively.

were removed from within each of the small squares. Within two hours of this impact event, all sixteen sampling quadrats were re-sampled to measure the post-impact community state.

Mosaic construction

Image mosaics of the sampling quadrats were constructed from the stills and video imagery using a similar method to that of Martin & Martin (2002); these workers created a continuous 25 m^2 image of the seabed by manually mosaicing 25 × 1 m^2 images collected from a stills camera and housing in a self-erecting bipod passed over ground-control points, provided by a 5 × 5 m aluminium frame constructed *in situ*. Our method differed in the scale of application and in the capture of images, as outlined above. There were additional differences regarding the collection of video imagery.

To extract video image tiles, the video imagery was played in Microsoft Windows Movie Maker and, using a freeze-frame function, the sharpest image from each of the 16 25-×-25-cm areas of the 100-×-100-cm sampling quadrat was extracted as a stills image tile at an image resolution of 96 dots per inch (dpi). This resolution was the maximum achievable using the video footage and software available. From this point onward all image tiles, from both the DSLR camera and the digital handycam video camera, were treated identically, using Adobe Photoshop CS2. Images were cropped to fit the 25 × 25 cm quadrat. Subsequently, image tiles were sharpened and the colour balance enhanced (where necessary), before they were manually mosaiced into one composite image of the entire 100 × 100 cm sampling quadrat. Due to the nature of the medium, images from the DSLR camera were saved at a maximum resolution of 300 dpi (Figure 1).

Data extraction techniques

Two types of data were collected from each image, one to represent the community's morphological composition (community structure hereafter) and the other its unique assemblage of taxa (community composition hereafter). For community structure, species were assigned to broad benthic categories of coarse taxonomic resolution to represent their structural role within the community, and recorded as percentage cover of the entire sampling quadrat area. The groups selected were: red foliose algae >1 cm height above the substratum (RF); non-red foliose algae >1 cm height above the substratum (AF); red algal turf <1 cm height above the substratum (RT); non-red algal turf <1 cm height above the substratum (AT); crustose coralline algae (CCA); mixed hydrozoan/bryozoan/algal turf <1 cm height above the substratum (T); and bryozoans (BRY). To represent the community composition, taxa were identified to the highest taxonomic resolution possible from the imagery and recorded as present or absent. This latter data set resulted in the compilation of a species list for each sample. These two types of data were extracted from each of the stills mosaics, by a single observer, using three different data-extraction techniques in the following order: visual estimation of cover; visual estimation of cover by frequency of occurrence; and point-intercept cover estimation. This order followed the level of data density of each technique, from high to low, respectively. Data were extracted from all 64 quadrats using only one technique first (16 pre- and post-impact stills mosaics; 16 pre- and post-impact video mosaics), before the use of the next. This sufficiently reduced any observer bias because the volume of quadrats was great enough to ensure the observer had no memory of the previous 64 quadrats, before the next data extraction technique was employed. As the observer was sufficiently familiar with the species and broad benthic categories under investigation, learning effects were also considered negligible.

VISUAL ESTIMATION OF COVER (ESTIM)

The ESTIM technique is a well-established method used worldwide, where the observer 'says what they see' (Dethier *et al.*, 1993; Leujak & Ormond, 2007). Among the three techniques tested it was considered the most accurate, and therefore, the most reliable in terms of providing the best estimate of the 'real' community structure and composition (Dethier *et al.*, 1993; Beaumont *et al.*, 2007; Leujak & Ormond, 2007). Therefore, data from this technique provided a baseline against which the other techniques were compared. In this study, biota within the entire image area were identified and recorded as present or absent to represent the community composition of each sample. Then a visual estimation of the percentage cover of each benthic category was conducted using the observer's judgement, to represent the community structure of each sample. Only the ESTIM technique was used to extract community structure and composition data from the video mosaics. The other techniques could have been applied to these mosaics, but differences between these data and those derived from the higher resolution stills imagery would be more readily detected by the most accurate and reliable technique: ESTIM. Therefore, ESTIM was the only technique used for the comparison of data collected by stills and video media.

VISUAL ESTIMATION OF COVER BY FREQUENCY OF OCCURRENCE (FREQ)

The FREQ technique differs from ESTIM in that it requires the use of a sampling grid to determine percentage cover (US Fish and Wildlife Service, 2004; Beaumont *et al.*, 2007). The community structure was extracted from the mosaics by overlaying a grid of 100 equal-sized squares, each measuring 10 × 10 cm, from which the occurrence of benthic categories within each square was noted as either present or absent. Regardless of whether a benthic category occupied the whole or only a small portion of the square, it was still recorded as occurring in 1% of the image area. The same categories were also recorded twice if they happened to occupy two adjacent squares. Community composition was extracted in the same manner as it was for the ESTIM technique.

POINT-INTERCEPT COVER ESTIMATION (POINT)

The POINT technique is another widely-used technique, favoured for its ease and speed of application (Aronson *et al.*, 1994; Preskitt *et al.*, 2004; Jokiel *et al.*, 2005). Images are typically overlaid with pre-determined number of points, either randomly or evenly spaced, and data are extracted from each of these points only. In this study, the community structure was determined from 100 evenly spaced point-intercepts spread across each mosaic. The benthic category under each point was recorded to represent 1% of the image area. If taxa were present under any of the point-intercepts, they were recorded as being present, thus generating the community composition of that sample. Evenly spaced points were chosen over random points for their ease of application to data extraction (Drummond & Connell, 2005). Individual rarefaction curves constructed for each quadrat in this study confirm that the horizontal asymptote was consistently approached using 100 points per image. Because little improvement in species detection was gained by increasing the number of points per image, 100 points was justified for this study.

Data analysis

Four factors were considered in the experimental design of this investigation: DEPTH (8 m, 14 m, 18 m, 22 m), MEDIUM (stills, video), TECHNIQUE (ESTIM, FREQ, POINT) and IMPACT (pre-impact or post-impact). These factors were considered fixed as each was deliberately chosen for manipulation and not randomly selected. To minimize issues associated with distributional assumptions of conventional analysis of variance, and to take advantage of the multivariate nature of the community data, all hypotheses were tested using the PERMANOVA routine in PRIMER v6.1.12 (Anderson, 2001; Anderson *et al.*, 2008). The first series of tests compared ESTIM data acquired from the stills and video imagery by investigating trends in community structure and composition between the factors DEPTH and MEDIUM. The similarity of percentages routine (SIMPER) was employed to further explore differences between the community compositions of the stills- and video-derived data. A second PERMANOVA test compared stills data acquired by the different data-extraction techniques by investigating trends in community structure and composition between the factors DEPTH, IMPACT and TECHNIQUE. In both cases, a full factorial model was applied to the benthic category data, then to community composition data. Prior to calculating the Bray–Curtis resemblance matrix, the benthic category data were fourth-root transformed to even the spread of percentage cover by reducing the relative expression of the more dominant categories, while increasing the relative expression of the less dominant categories. Community composition data, however, required no such transformation as they were already in presence/absence format. In each case, PERMANOVA was run with 999 permutations. To visualize these analyses, we used non-parametric multidimensional scaling (nMDS) of the corresponding similarity matrices.

To establish which of the data collection media and extraction techniques were most efficient, the time taken to conduct the work using each approach was analysed. The field costs of SCUBA-diving surveys (boat hire, diver costs and training), basic equipment costs (SCUBA equipment and quadrat construction) and all software costs (Photoshop CS2 and PRIMER v6.1.12) were excluded from the analysis as they were common to all methods used in this study, and would therefore offer no basis for comparison. The subtle difference in cost between the two media (Figure 1A, B) was also excluded from the analysis as relative to the other operational costs in this study, this difference over the life of a monitoring programme would be negligible (Brown *et al.*, 2004; Leujak & Ormond, 2007). The analysis was divided into two sections: the time taken to complete various tasks (time effort) and the benefits of each approach. The following measures were used to evaluate benefit: image resolution; species richness, measured as the mean number of species per sample; and taxonomic benefit, measured as the calculated rate of species detection per unit time. Only pre-impact data were used in this analysis as IMPACT was not under investigation.

RESULTS

Comparison of data collection media

Sixteen comparative 100 × 100 cm image-mosaics were constructed from stills-imagery at an image resolution of 300 dpi, and from video-imagery at 96 dpi. PERMANOVA revealed that significant differences existed between the stills- and video-derived community structure and composition (Table 1). These differences were clearly visible in comparative nMDS biplots (Figure 2). In addition, all community structure and composition data changed significantly with survey depth (Table 1). Clear shifts in the community structure with increases in depth are evident from further observation of the nMDS ordination plots in Figure 2A. These shifts appear to be consistent among data collected from the different imagery, as confirmed by the non-significant interaction terms in the models (Table 1).

Careful inspection of the relative abundance of individual benthic categories at each depth (Figure 3) did not immediately reveal the differences between the data derived from the different imagery. However, univariate PERMANOVA by benthic category detected significant differences in the expression of the RT, AT, CCA and BRY benthic categories determined from the stills and video imagery (Table 2). These groups also had the lowest percentage cover of all groups in the data (Figure 3). There were no differences between the groups with the greatest expression of cover measured from the stills and video imagery: the RF, AF and T (Table 2). Therefore, the source of the overall difference in the community structure derived from the stills and video imagery appeared to be related to the expression of the less dominant benthic categories.

Further exploration of the community composition data revealed species richness to be approximately four-times higher among the stills-derived data than the video-derived data between survey depths (Table 3). SIMPER analysis of species which contributed towards 90% of the variability within each depth showed a gradual shift towards different assemblages with increases in depth among the stills-derived data. As a general observation, the majority of the contributing species identified in the stills imagery were not identified from the comparative video imagery. Those that were identifiable from low-resolution imagery included *Delessaria sanguinea* (Hudson), *Calliblepharis ciliata* (Hudson) and *Laminaria digitata* (Hudson). Species that require high-resolution imagery for identification were identifiable only in the stills imagery; these included *Plocamium cartilagineum* (Linnaeus), *Sphaerococcus coronopifolius* (Stackhouse) and *Caryophyllia smithii* (Stokes & Broderip).

Table 1. PERMANOVA results for a full factorial model of the fixed factors DEPTH and MEDIUM. Tests were conducted on (A) community composition and (B) species assemblage data determined by the ESTIM technique from comparative stills and video imagery. Bold results indicate a significant effect ($P \leq 0.05$).

Factor/term	A. Community structure						B. Community composition					
	df	SS	MS	Pseudo-F	*P*	perms	df	SS	MS	Pseudo-F	*P*	perms
DEPTH	3	1625.2	541.7	8.077	**0.001**	999	3	18778.0	6259.4	9.177	**0.001**	999
MEDIUM	1	794.9	794.9	11.851	**0.001**	999	1	23146.0	23146.0	33.935	**0.001**	998
DE × ME	3	141.8	47.3	0.705	0.654	997	3	2527.8	842.6	1.235	0.285	999
Residual	24	1609.8	67.1				24	16370.0	682.1			
Total	31	4171.7					31	60822.0				

df, degrees of freedom; SS, sums of squares; MS, mean sums of squares; perms = permutations; DE, DEPTH; ME, MEDIUM.

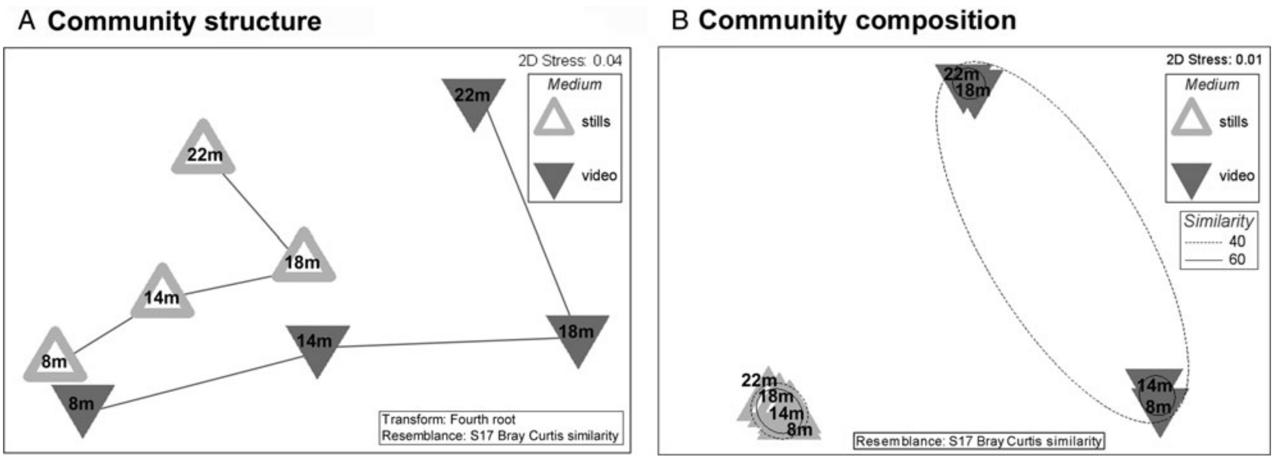

Fig. 2. Comparative non-parametric multidimensional scaling ordination plots of stills and video data, to show similarities in (A) fourth-root transformed community structure, and (B) presence/absence community composition data across depth. Each projected point represents the average of four replicates, i.e. the centroid, from each depth.

Comparison of data extraction techniques

PERMANOVA revealed that the community structure and composition determined by the techniques were different (Table 4). The community structure and composition also changed with increases in depth (Table 4), as previously seen (Table 2; Figure 2). Dissimilarity in the impressions of community structure and composition determined by the techniques are clearly visible in comparative nMDS ordinations (Figure 4).

Further data exploration revealed similarities between the impressions of community structure determined by the ESTIM and POINT techniques (Figure 4A). Pairwise comparisons revealed these similarities to be significant among data from 8 and 22 m depth, but not among those from 14 and 18 m depth (Table 5A). At these latter depths, dissimilarity between the expressions of RF at 14 m ($t = 5.376$, $P = 0.002$, $df = 12$), and of RT ($t = 2.724$, $P = 0.019$, $df = 11$), T ($t = 2.872$, $P = 0.015$, $df = 12$) and BRY ($t = 4.826$, $P = 0.004$, $df = 11$) from 18 m, likely drove the differences between the ESTIM- and POINT-derived community structure. In contrast to these techniques, impressions of community structure determined by the FREQ technique were consistently different from the other techniques at all depths (Table 5A).

A careful examination of the expression of the individual benthic categories between the depths highlights the similarities and dissimilarities among the impressions of community structure determined by the different techniques (Figure 5). The benthic categories recorded by the ESTIM and POINT techniques are expressed to similar percentage covers (Figure 5A, C). Only close inspection of the categories reveals the slightly higher estimates of the RF category, and slight under-expression of the turf categories (AT, RT and T). However, these differences are significant only at 14 and=18 m, as previously described. Data collected using the FREQ technique provide substantially higher estimates of each benthic category, relative to those of the other techniques (Figure 5B). This was apparent among the categories that scored percentage covers of <20% using the other techniques: AF, AT, RT, T, CCA and BRY. This 'over-expression' effect resulted in a unique impression of community structure using the FREQ technique, relative to the others.

Figure 5 also shows the effects that the experimental removal (10% of the macroalgae from the sampled areas) had on the impression of community structure: a reduction in the expression of RF and AF categories (canopy-forming groups), and an increase in the expression of RT, AT, T and CCA categories (understorey groups). The lack of significant

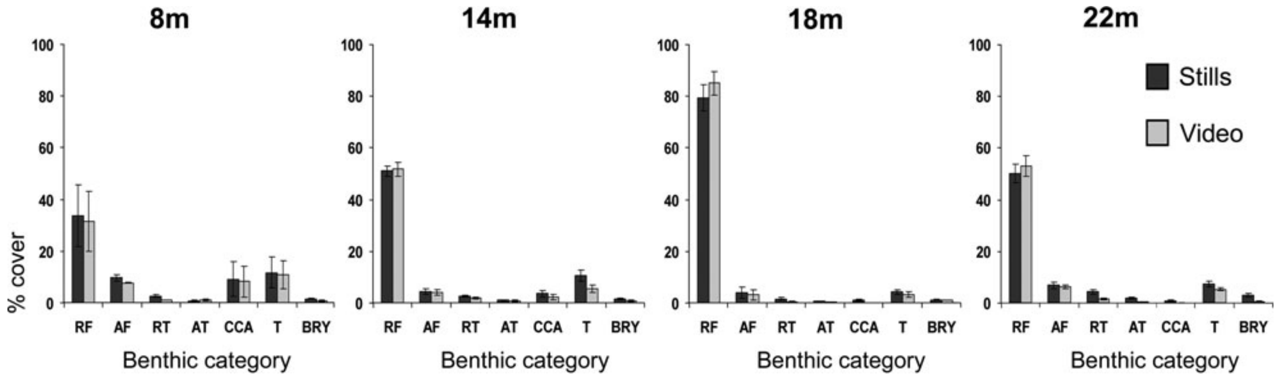

Fig. 3. Comparative plots of mean percentage cover of benthic categories determined from stills and video imagery across depth (as indicated). Error bars show standard error of the mean. Categories are red foliose algae >1 cm height above substratum (RF), non-red foliose algae >1 cm height above substratum (AF), red algal turf <1 cm height above substratum (RT), non-red algal turf <1 cm height above substratum (AT), crustose coralline algae (CCA), mixed hydoid and algal turf <1 cm height above substratum (T) and bryozoan (BRY).

Table 2. PERMANOVA results of fitting a full factorial model to the fixed factors DEPTH and MEDIUM. Tests were conducted on individual benthic categories determined by the ESTIM technique from comparative stills and video imagery. Significance is indicated by asterisks: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

Factor/ term	df	Benthic category						
		Pseudo-F values						
		RF	AF	RT	AT	T	CCA	BRY
DEPTH	3	4.84**	1.85*	8.44***	2.70	3.16*	17.08***	0.29
MEDIUM	1	0.01	0.74	24.16***	13.36**	2.52	16.44***	283.70***
DE × ME	3	0.02	0.78	1.03	1.95	0.40	13.40***	0.29

RF, red foliose algae; AF, non-red foliose algae; RT, red algal turf; AT, non-red algal turf; CCA, crustose coralline algae; T, mixed hydoid and algal turf; BRY, bryozoan; df, degrees of freedom; DE, DEPTH; ME, MEDIUM.

interactions involving impact indicates that these trends were similar across all depths and techniques, and this is supported by visual representations of the data (Figures 5 & 6). Despite these observations, *post-hoc* testing with PERMANOVA showed that a significant shift in community structure, as a result of the experimental impact, was detected only in the community structure determined by the POINT technique ($t = 1.772$, df $= 24$, $P = 0.029$); ESTIM ($t = 1.455$, df $= 24$, $P = 0.135$) and FREQ ($t = 1.264$, df $= 24$, $P = 0.187$) failed to detect this change. This showed that by using 16 pre- and post-impact replicates alone, only the POINT technique collected community structure data sensitive enough to react to the 10% change in macroalgal cover. Only when the model was refitted to ignore non-significant interaction terms (IMPACT × TECHNIQUE), could the other techniques collect data sensitive enough to react to the experimental change. In this instance, the ESTIM and FREQ techniques used between 17 and 48 replicates to detect the changes that the POINT technique achieved using 16 replicates.

There was no such change in the community composition determined by any of the techniques due to the experimental impact (Table 4B), despite apparent visual dissimilarity from their two-dimensional ordination (Figure 4B). However, the community composition determined by the techniques did change with increases in depth (Table 4B). Indeed, a gradual shift in each community composition is evident from their two-dimensional ordination, and is common among all data derived by the different techniques (Figure 4B). Regarding the effect of the techniques themselves, pairwise comparisons showed that the community composition determined by the ESTIM and FREQ techniques were indistinguishable and differed significantly from those determined by the POINT technique (Table 5B; Figure 4B). From comparison of the species richness determined by each technique at each depth, the relatively poor expression of species in the POINT data clearly drove the differences with respect to the other techniques (Figure 6).

Efficiency analysis of media and techniques

The image mosaicing procedure took 15 minutes per m^2 of seabed sampled for both stills and video imagery alike (Table 6). All stills imagery from the sampling quadrats were collected and processed at the same time. It was fractionally quicker for the divers to collect the video imagery, but it took longer to process it in the laboratory because of the additional still-image tile-extraction process. Despite the extra processing time, the total effort of collecting, processing and extracting data from the video imagery was the lowest in this study (39 min.quadrat^{-1}). However, the lowest species richness values were also observed from the video imagery (6 sp.quadrat^{-1}; stills had 21 sp.quadrat^{-1}). As a result, data extracted from the video imagery had the poorest taxonomic benefit of any approach in this study (0.15 sp.min^{-1}).

The relative efficiencies and benefits of the data-extraction techniques varied because of differences in data-extraction times and species richness values (Table 6). Overall, the POINT technique extracted community composition and species assemblage data most efficiently (41 min.quadrat^{-1}), owing to the inherent speed of data extraction using only 100 points per image-mosaic. Similar to the video-derived data in this respect, the POINT-derived data also had the lowest species richness values (11 sp.quadrat^{-1}) and taxonomic benefit (0.26 sp.min^{-1}) among the techniques used to extract data from stills images. In contrast with video-derived data, however, the POINT technique had a comparatively higher taxonomic benefit than that from the video imagery (0.26 sp.min^{-1} versus 0.15 sp.min^{-1}, respectivly). The highest taxonomic benefits were observed among the ESTIM and FREQ technique data (0.37 and 0.36 sp.min^{-1}, respectively). This was likely due to the 100% spatial coverage of high-resolution imagery employed in the data-extraction method of these techniques. These techniques also required a similar amount of total effort (57 and 58 min.quadrat^{-1}, respectively), which were the highest of all approaches in this study. It is likely that this poor efficiency, relative to the other approaches, was the main cost that balances the high taxonomic benefits.

DISCUSSION

Few studies have tackled such a diverse array of factors in one investigation (Drummond & Connell, 2005; Jokiel *et al.*, 2005; Beaumont *et al.*, 2007; Leujak & Ormond, 2007), and none have simultaneously tackled the question of survey depth, data acquisition medium, data extraction technique and experimental impact detection. Although all four goals of this study were achieved, issues raised in the results have highlighted a few methodological concerns that warrant further discussion.

Image-mosaicing using stills and video cameras

Leujak & Ormond (2007) found that in order to fit a 1 m^2 area into one image with a 28 mm lens (similar to that used in this

Table 3. Comparative mean species richness (presence/absence) and SIMPER results of 90% species that contributed to community composition determined from (A) stills and (B) video imagery across different depths (as indicated). Values represent the aggregation of four replicates from each depth.

(A) stills	8 m	14 m	18 m	22 m
Mean species richness (± SE)	17.8 (0.9)	21.0 (1.4)	19.0 (1.9)	25.5 (1.0)
SIMPER average similarity (%)	55.2	69.1	70.2	67.3
Taxa	**Contb.(%)**	**Contb.(%)**	**Contb.(%)**	**Contb.(%)**
Crisea sp.	10.2	6.9	7.6	5.8
Delessaria sanguinea	10.2	6.9	7.6	5.8
Heterosiphonia plumosa	10.2	6.9	7.6	5.8
Electra pilosa	10.2	6.9	7.6	3.0
Plocamium cartilagineum	5.0	6.9	7.6	5.8
Dictyota dichotoma	1.7	6.9	7.6	5.8
Bonnemaisonia asparagoides	5.0	3.5	7.6	5.8
Calliblepharis ciliata		6.9	7.6	2.9
Caryophyllia smithii	1.7	3.5		5.8
Cryptopleura ramosa	1.6		3.6	3.0
Sphaerococcus coronopifolia			7.6	5.8
Halarachnion ligulatum			7.6	5.8
Odonthalia dentata	5.3	6.9		
Acrosorium venulosum			3.6	5.8
Drachiella spectabilis	5.1			3.0
Halopteris filicina			3.6	5.8
Callophyllis lacinata	1.6	6.9		
Alcyonidium diaphanum	1.8	6.9		
Palmaria palmata	5.3	3.5		
Echinus esculentus	5.0	3.5		
Laminaria digitata	5.3			
Gibbula cinerea	5.1			
Ahnfeltia plicata		3.6		
Pomatoceros triqueter		3.2		
Parasmittina trispinosa			3.6	
Dictyopteris membranacea				5.8
Nemertesia antennina				3.0
Sabella pavonina				3.0
Sporochnus pedunculatus				2.9
Total contributrion (%)	90.4	90.1	90.2	90.7

(B) video	8 m	14 m	18 m	22 m
Mean species richness (± SE)	4.5 (1.0)	7.0 (0.0)	5.5 (0.5)	5.8 (1.3)
SIMPER average similarity (%)	54.9	76.2	75.6	61.6
Taxa	**Contb.(%)**	**Contb.(%)**	**Contb.(%)**	**Contb.(%)**
Electra pilosa	17.3	9.4	24.3	3.4
Calliblepharis ciliata		18.8	24.3	30.0
Delessaria sanguinea	17.3	18.8	24.3	
Laminaria digitata	42.9	18.8		
Echinus esculentus	17.3	18.8		
Dictyota dichotoma		9.4		30.0
Dictyotoma membrancea			11.0	30.0
Halarachnion ligulatum			12.5	
Total contributrion (%)	94.9	93.8	96.3	93.2

study), stills imagery had to be collected from approximately 2 m above the substratum. At this camera altitude in all but the clearest waters, however, it would be difficult to identify even larger benthic organisms and coarse benthic categories (Barrett *et al.*, 2007). As a goal of this study, the mosaicing method accounted for potential turbidity by reducing the distance between the camera lens and the substratum to only 40 cm. Consequently, areas smaller than 1 m^2 were sampled, but owing to the mosaicing process used to join these areas together, uninterrupted estimation of benthic cover per square metre was still achieved. In addition, fine-scale (<1 cm) taxonomic features of small animals (~1 cm size), such as *Caryophyllia smithii*, and macroalgal species with small and delicate taxonomic features, such as *Plocamium cartilagineum* and *Sphaerococcus coronopifolius*, were detected due to the high-resolution imagery and this contributed towards the high species richness determined from the stills mosaics. It is unlikely that these species would have been detected if a single 1-m photoquadrat was collected instead of the mosaiced 25-×-25-cm quadrats.

In contrast to the stills mosaics, those made from the video imagery did not benefit as much from using the mosaicing method. Because of inherently lower image resolution, only around a quarter of the species identified from the stills

Table 4. PERMANOVA results of fitting a full factorial model to the fixed factors DEPTH, IMPACT and TECHNIQUE. Tests were conducted on (A) community composition and (B) species assemblage data determined by different data-extraction techniques from stills imagery. Bold results indicate a significant result ($P \leq 0.05$).

Factor/term	(A) Community structure						(B) Community compositions					
	df	SS	MS	Pseudo-F	*P*	perms	df	SS	MS	Pseudo-F	*P*	perms
DEPTH	3	2816.9	939.0	13.631	**0.001**	999	3	37254.0	12418.0	16.637	**0.001**	996
IMPACT	1	314.7	314.7	4.568	**0.005**	999	1	420.2	420.2	0.563	0.784	998
TECHNIQUE	2	11378.0	5688.8	82.584	**0.001**	999	2	12817.0	6408.7	8.586	**0.001**	999
DE × IM	3	91.4	30.5	0.442	0.858	999	3	1053.0	351.0	0.470	0.965	999
DE × TE	6	1435.3	239.2	3.473	**0.001**	998	6	7080.4	1180.1	1.581	**0.025**	998
IM × TE	2	247.3	123.6	1.795	0.086	998	2	409.5	204.7	0.274	0.988	998
DE × IM × TE	6	94.6	15.8	0.229	0.992	999	6	134.3	22.4	0.030	1.000	998
Residual	72	4959.8	68.9				72	53741.0	746.4			
Total	95	21338.0					95	112910.0				

df, degrees of freedom; SS, sums of squares; MS, meam sums of squares; perms = permutations; DE, DEPTH; IM, IMPACT; TE, TECHNIQUE.

mosaics were recognized in the video mosaics. Fewer species are often identified from low-resolution imagery, such as that acquired from underwater video cameras (Leujak & Ormond, 2007) or low-resolution stills cameras (Cabaitan *et al.*, 2007). Image mosaics using such media may not have the same benefits as those constructed from high-resolution stills imagery. For example, Lirman *et al.* (2007) report difficulty in accurately identifying coral colonies <4 cm across from video mosaics of 400 m^2 areas of tropical seabed. However, the main advantages of using video cameras for benthic community monitoring are that data are rapidly and cheaply acquired when diver time is expensive (Leonard & Clark, 1993; Lirman *et al.*, 2007). Furthermore, at coarser taxonomic resolutions (i.e. benthic categories), video has proven to be an efficient and effective benthic monitoring tool (Aronson *et al.*, 1994; Brown *et al.*, 2004; Parsons *et al.*, 2004; Houk & Van Woesik, 2006, Cabaitan *et al.*, 2007; Leujak & Ormond, 2007). For these reasons, video has proven an ideal tool for making mosaics of large areas (e.g. 400 m^2) of seabed where relatively coarse taxonomic work (e.g. tropical hard-coral cover assessments) and general assessments of ecosystem health (e.g. boat damage to tropical coral-reefs) are of concern (Lirman *et al.*, 2007, 2010). In this study, however, for similar cost, efficiency and scale of operation (1 m^2) it was more beneficial to construct the mosaics from high-resolution stills imagery. As the collection of fine-scale data (~1 cm^2) was possible from only the stills imagery, the coverage of benthic categories comprising small organisms were more accurately estimated and more species were identified from each mosaic as a direct consequence.

A few issues with the mosaicing process require further consideration. Lens effects were observed in a few tiles of the stills image mosaics. The very slight degree of lens-related radial distortion (Choi *et al.*, 2006) can be reduced by placing the sampling quadrat on flat, even substrata, or if this is unavoidable then measurements of uneven substrata should be recorded and factored into any image correction software. The type of community must also be factored into any investigation using photoquadrats. A community with a canopy structure will likely obscure understorey taxa, making it difficult to collect data across the entire assemblage (Pech *et al.*, 2004). In addition, movement of algal fronds with tidal surge and current will influence which species are captured in the image at the time the photograph is taken, which in turn will affect the overall percentage cover and detection of certain taxa, particularly understorey taxa. In using optical

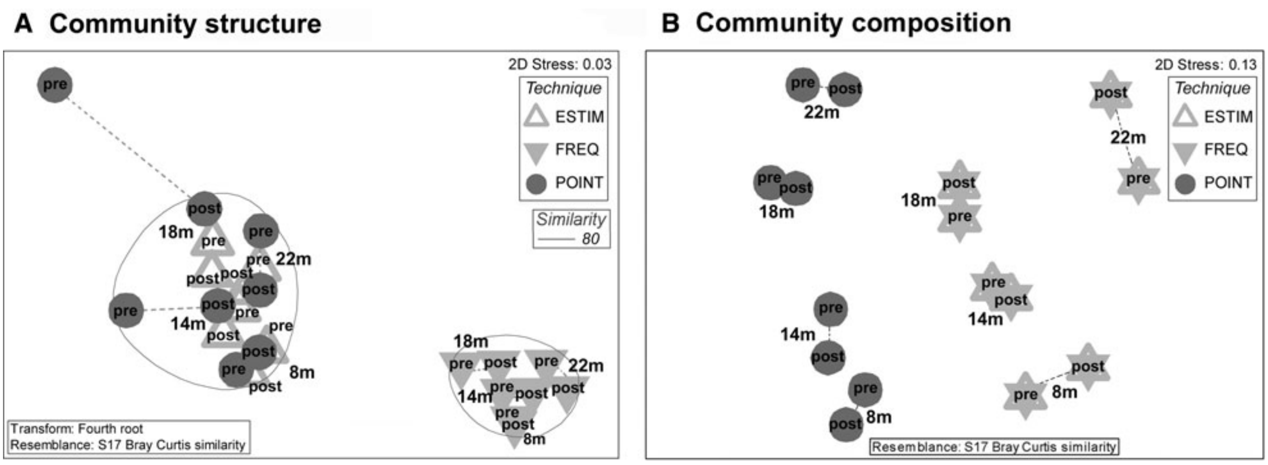

Fig. 4. Comparative non-parametric multidimensional scaling ordination plots of community data determined using different data extraction techniques, showing similarities between impact condition and depth of (A) fourth-root transformed community structure and (B) presence/absence community composition data (as indicated). Data are averaged to represent the mean of four replicates, i.e. centroids. Dotted lines link the same group of samples across different impact conditions.

Table 5. PERMANOVA pairwise comparison test results using the term DEPTH × TECHNIQUE. Tests compared (A) community structure and (B) species assemblage data between the extraction techniques from different depths (as indicated). Significance is indicated by asterisks: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

Source data	Groups	df	*t* values			
			8 m	14 m	18 m	22 m
(A) Community structure	ESTIM, FREQ	12	4.17***	11.57***	7.43***	13.44***
	ESTIM, POINT	12	0.56	2.73**	2.97***	1.30
	FREQ, POINT	12	3.76***	6.72**	5.76**	9.69**
(B) Community composition	ESTIM, FREQ	12	0.00	0.00	0.00	0.00
	ESTIM, POINT	12	1.82	2.23***	2.28**	2.96**
	FREQ, POINT	12	1.82	2.23***	2.28**	2.96**

sampling methods on this type of subtidal community, these problems are unavoidable and therefore must be considered as a standard problem (Leonard & Clark, 1993), whether using image mosaics or just single images.

Data extraction techniques

The comparison of community data collected by the different data extraction techniques highlighted the various attributes of each technique. The additional evaluation of benefit and efficiency indicated those attributes which could be considered advantageous or disadvantageous to benthic monitoring using image mosaics. The accuracy of community cover estimates were evaluated relative to the estimates provided by the ESTIM technique. Although this technique was more subject to observer bias than the others (Meese & Tomich, 1992; Benedetti-Cecchi *et al.*, 1996), it has been shown that with sufficient training, even inexperienced observers can use it to collect data that are statistically indistinguishable from that collected by experienced observers (Dethier *et al.*, 1993). Without any additional means of assessing accuracy in this study, the best estimate of the 'real' community structure and composition was therefore that recorded using the ESTIM technique (Dethier *et al.*, 1993; Beaumont *et al.*, 2007; Leujak & Ormond, 2007). Furthermore, in having the greatest taxonomic benefit of all techniques, it can also be considered the most reliable and efficient at extracting species data from the 1 m^2 image-mosaics.

The other techniques, FREQ and POINT, estimated community structure using pre-determined data-extraction measures that did not rely on the observer's judgement of percentage cover of benthic categories. The relative observer-objectivity gained through using frequency-grid and point-intercept extraction measures is of much benefit to monitoring programmes that utilize multiple observers over their lifetime (Bohnsack, 1979; Brown *et al.*, 2004; Jokiel *et al.*, 2005). Despite the inherent differences in data extraction method, the impressions of community structure determined by the POINT and ESTIM techniques were remarkably similar. The POINT technique, however, clearly outperformed the others in terms of efficiency: community structure data were consistently extracted in half the time it took for the other techniques. Altogether, the speed, consistency and relative objectivity were the clearest advantages of using the POINT technique to extract community data. These attributes are not uncommon in other marine studies (Aronson *et al.*, 1994; Drummond & Connell, 2005; Jokiel *et al.*, 2005), where point-based data extraction techniques are also considered to be 'simple, quick and inexpensive' for work at coarse taxonomic resolutions (Aronson *et al.*, 1994). These attributes could be of advantage to monitoring work, where methods that are efficient and cost-effective are usually favoured (Brown *et al.*, 2004; Leujak & Ormond, 2007).

However, the POINT technique did have one distinct disadvantage relative to the others: low data density per unit area (Drummond & Connell, 2005). As a result, species richness was underestimated relative to the other techniques. A similar effect is reported in other studies (Meese & Tomich, 1992; Dethier *et al.*, 1993; Beaumont *et al.*, 2007). There is evidence to suggest that by increasing the number of point-intercepts per sampling area the effect is reduced and more species recorded. However, this comes at the expense of data extraction efficiency (Meese & Tomich, 1992; Dethier *et al.*, 1993). Alternatively, a reduction in quadrat size would increase the density of data extraction points per quadrat and achieve a similar effect, although there would be a consequent reduction in the area of seabed sampled. As the implementation of either of these measures could reduce the benefits of using the image mosaics, the POINT technique is therefore, not the ideal data extraction technique for use on 1 m^2 photo-mosaics. Alternatively, it would be more suited to the collection of coarse community data from smaller photo-quadrats, where large numbers of replicates may be collected and data extracted efficiently, objectively and cost-effectively.

Despite the relative lack of application of the FREQ technique in previous benthic monitoring studies, this technique offers three potential benefits. First, this method of quantifying community structure can be used for colonial and solitary species simultaneously (freq./unit area), whereas percentage cover estimations (%/unit area) and abundance counts (no./unit area) are typically conducted across different scales (Beaumont *et al.*, 2007). Secondly, Bohnsack (1979) noted that its high degree of objectivity offered distinct advantages regarding observer bias, similar to that of the POINT technique. Thirdly, the relative over-expression of less-dominant benthic categories could make any subtle changes to community structure more readily detectable in a long-term benthic study. However, the over-expression effect also had its disadvantages. In this study, gross differences in community structure determined by the ESTIM and FREQ techniques signified that the latter generated the least accurate data of all techniques under investigation. In addition, these data showed the highest variability of all techniques tested. Therefore, despite having an efficiency, taxonomic benefit and species richness comparable to that of the ESTIM technique, the inherent overestimation effect and subsequent variability of cover estimations made

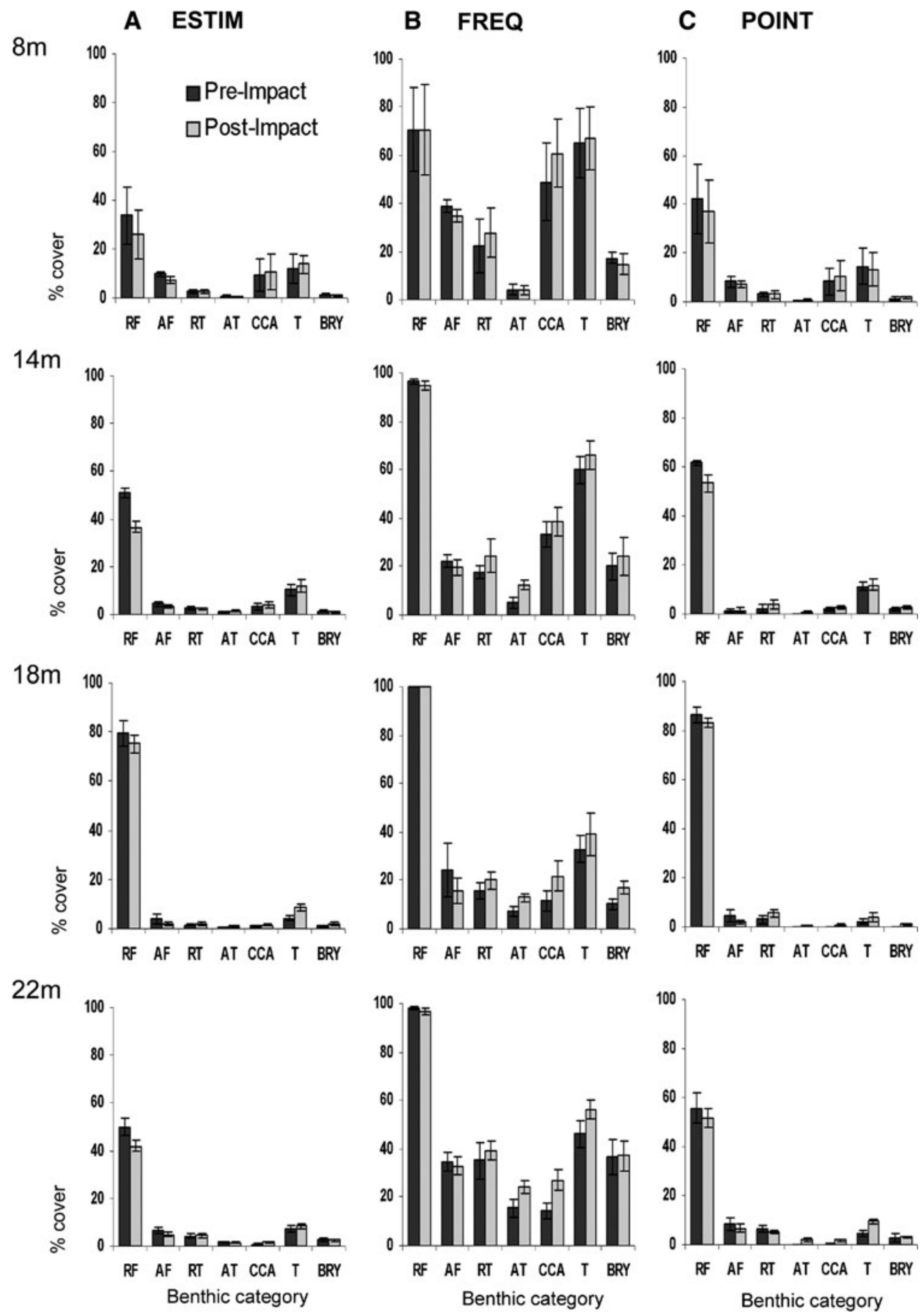

Fig. 5. Comparative plots of mean percentage cover of benthic categories between pre- and post-impact conditions across depth (as indicated). Plots are made from community composition data extracted using (A) ESTIM, (B) FREQ and (C) POINT techniques on comparative stills imagery (error bars show standard error of the mean). Categories are red foliose algae >1 cm height above substratum (RF), non-red foliose algae >1 cm height above substratum (AF), red algal turf <1 cm height above substratum (RT), non-red algal turf <1 cm height above substratum (AT), crustose coralline algae (CCA), mixed hydoid and algal turf <1 cm height above substratum (T) and bryozoan (BRY).

by the FREQ technique make it unsuitable for monitoring use on the 1 m^2 photo-mosaics.

Change detection

A monitoring method must be able to register changes to the community over time in order to be effective (Brown *et al.*, 2004; Jokiel *et al.*, 2005). By simulating such community change through experimental impact, we were able to assess each technique's response and ability to detect community change. As could be expected, a reduction in canopy-forming categories (RF and AF) and a general increase in the expression of understorey categories (RT, AT, T and CCA) were observed. However, only the data collected by the POINT technique proved sensitive enough to detect these changes using 16 replicate samples. In addition to the previously outlined advantages of this technique, this sensitivity to change constitutes an additional advantage of the POINT

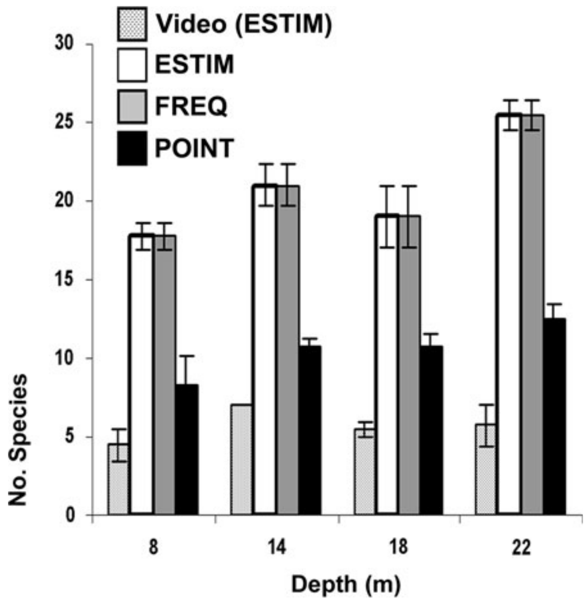

Fig. 6. Comparative plot of mean number of species per depth determined by different media and data extraction techniques (as indicated). ESTIM, FREQ and POINT data were acquired from stills imagery alone, unless indicated. Error bars show standard error.

technique. In considering that the ESTIM and FREQ techniques required between 17 and 48 replicates to detect same experimental change (when non-significant interaction terms were removed from the statistical model), such levels of replication are prohibitively high to be used in regular monitoring (Leujak & Ormond, 2007). For these techniques perhaps a 10% change in community structure was an unrealistic monitoring target. Indeed, few studies have designed methods which can confidently detect a 10% change in the relative cover of benthic categories (Brown *et al.*, 2004; Jokiel *et al.*, 2005). In maintaining the balance between practicality and sensitivity, experimental impacts to 20–50% of the community occupying the substratum could have yielded more useful estimates of change and the replication necessary to detect that change (Leujak & Ormond, 2007). For certain though, the analytical approach used in this study highlights the statistical power and flexibility of PERMANOVA when used in conjunction with a robust experimental design.

Table 6. Time required and benefits of the different media and techniques in this study. (A) Time effort is measured in minutes and is standardized to the time taken to complete one complete quadrat (min/quadrat); (B) benefits are represented by image resolution, mean species richness and taxonomic benefit index (number of species detected per unit time) and are standardized to units per quadrat. Additional units are indicated in parentheses.

Medium	**Video**	**Stills**		
Technique	**ESTIM**	**ESTIM**	**FREQ**	**POINT**
(A) Time (min.quadrat^{-1})				
Sampling effort	4.00	5.00	5.00	5.00
Data processing effort	10.00	5.00	5.00	5.00
Image mosaic effort	15.00	15.00	15.00	15.00
Data extraction effort (±SD)	10.19	31.29	32.63	16.00
	(4.23)	(8.08)	(7.83)	(2.80)
Total effort	39.19	56.29	57.63	41.00
(B) Benefit (quadrat^{-1})				
Image resolution (dpi)	96.00	300.00	300.00	300.00
Mean species richness (±SD)	5.68	20.88	20.88	10.56
	(1.82)	(3.38)	(3.38)	(2.58)
Taxonomic benefit (sp.min^{-1}·quadrat^{-1})	0.15	0.37	0.36	0.26

Conclusions and recommendations

The robust design of our experiment, the power of PERMANOVA and the findings of similar methodological studies (Dethier *et al.*, 1993; Benedetti-Cecchi *et al.*, 1996; Brown *et al.*, 2004; Bowden, 2005; Drummond & Connell, 2005; Jokiel *et al.*, 2005; Leujak & Ormond, 2007; Sayer & Poonian, 2007) facilitate sound recommendations regarding the relative efficiency and benefit of approaches tested in this study:

1. the image-mosaicing method developed in this study could be applied to fixed-station monitoring of communities, which demands high-resolution sampling approaches to assess fine-scale processes such as recruitment, growth and mortality of individuals (Brown *et al.*, 2004);
2. a recurring result throughout this investigation has been the difference between the impressions of the infralittoral communities at different depths. Although the experimental depths differed only by a few metres, the communities at those depths were statistically different. Because this was the case, the planning and design of any monitoring programme should carefully consider depth gradients with regard to results. Ideally, samples from the same depth should be appropriately replicated for greater robustness of data over time; and
3. although inappropriate for the image mosaics of this study, application of the POINT technique is recommended for use on high-resolution stills imagery collected from smaller quadrats, such as from the 25 × 25 cm photoquadrat used in this study. This approach could yield benthic community data efficiently and cost-effectively that prove sensitive to community change.

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