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Abundance estimation of rocky shore invertebrates at small spatial scale by high-resolution digital photography and digital image analysis

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Abstract

We have tested both the usefulness of high-resolution digital photography for data acquisition and digital image analysis, by non-supervised classification and high pass filter, for recognition and abundance estimation of benthic intertidal organisms. These digital tools were compared with visual scan and photo quadrat conventional methods. The comparison was done using 40 quadrats (10 × 5 cm) randomly selected along a 5-m transect on the rocky shore of the Pemaquid Point, Maine, USA. ANOVA for repeated measures was used to test differences among methods. Monte Carlo simulation analysis was used to explore differences among methods over a large set of data ($n = 100$, 500, 1000 quadrats). Differences among methods were observed when 40 quadrats were used. Tukey multiple comparison test showed that abundance estimation from visual scan, photo quadrat and digital image analysis by high pass filter do not differ significantly among them but differ from non-supervised classification results. Due to its accurate estimation, high pass filter (Prewitt) method was chosen as the most reliable digital method to estimate species abundance. Monte Carlo simulation of visual scan, photo quadrat and high pass filter results showed significant differences when the number of quadrats was larger. These results showed that the combined use of digital photography and digital image analysis techniques for the acquisition and analysis of recorded data is a powerful method for the study of intertidal benthic organisms. Results produced using these techniques

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were similar than those produced by conventional methods but were obtained in a much-reduced time.

Keywords: Benthos; Digital photography; Digital image analysis; Small spatial scale

1. Introduction

In nature, benthic organisms can be considered as distributed in systems hierarchically organized (Farmer and Adams, 1991). Traditionally, this structure can be viewed as fully nested, with higher levels representing assemblies of lower level units. Given this complexity, some researchers (e.g. Horne and Schneider, 1995; Somerfield and Gage, 2000) have focused their efforts on the development of new techniques and models that permit to simplify the approach used to understand properties of the different compartments of the hierarchical structure. There are still, however, numerous limitations to the understanding of scale and temporal dependent patterns in ecosystems. One of these limitations is a conceptual one because patches are often defined in some convenient but not necessarily biologically relevant manner in relation to the organisms being studied (Langton et al., 1995). Another limitation is related to the sampling methods used for acquiring and estimating biological information (Andrew and Mapstone, 1987).

Estimations of the accuracy and efficiency of the most common sampling methods used in benthic studies (e.g. visual scan, photo quadrats, video transects, point-contact, random dots) have been presented by different authors (e.g. Foster et al., 1991; Whorff and Griffing, 1992; Meese and Tomich, 1992; Miller and Ambrose, 2000). All these authors have reached the conclusion that these methods contain potential errors for estimating community properties of species (e.g. abundance, species diversity). For example, the introduction of variability due to the observer in the case of the visual method (Meese and Tomich, 1992) or under estimation of variables such as abundance or species richness due to the misidentification of species in the case of the photo quadrat or video transect methods.

In the last decade, new forms of multispectral sensors have been developed that has permitted the acquisition of digital imagery along the whole electromagnetic spectrum (King, 1995). This kind of technology has opened up new possibilities in the use of video and photographic cameras. Imagery acquired with these devices can be easily stored in standard computers at 3–4 s per frame with <20 cm of ground resolution. Therefore, it is possible now to acquire easily and at relatively low cost high quality digital field data allowing the built up of extensive image data bases to support qualitative and quantitative studies (Livingstone et al., 1999). Digital imagery and digital image analysis are now commonly used to obtain and interpret thematic maps in physical sciences and geophysics (Christakos, 2000). One typical and well-documented example is the study of the spatial distribution of laminated sediments (Dartnell and Gardner, 1993; Glasbey and Horgan, 1995; Cooper, 1997) and soils (Petterson et al., 1993).

The implementation of these tools to study marine benthos has not been fully explored. The utilization of digital techniques to sample rocky shores benthos could help to improve the results obtained by standard sampling methods like visual scan or photo quadrats. Since images are usually obtained in multispectral mode (red, green and blue), it is possible using different procedures of images analysis to eliminate radiometric distortion caused by panoramic effects like shadows and changes in rock surface coloration, bringing in turn both more accurate species identification and better estimates of the ecological properties of community (e.g abundance).

Up till now, digital image analysis has been used to map benthic assemblages and habitats at spatial scales from m to km applying principal component analysis (Pasqualini et al., 1997) and/or non-supervised and supervised classification methods (Sheppard et al., 1995; Cuevas-Jiménez et al., 2002). Digital image analysis was also used to study Antarctic benthic communities using landscape indices and principal component analysis (Teixidó et al., 2002). However, all images used in the studies cited above were acquired by analogue photography and digitized afterwards. The use of high-resolution digital photography in the study of marine benthos has not been yet reported. The aim of this paper is to examine the advantages offered by this technology for extracting at small spatial scales (cm to m) biological information (abundance, species richness). The results show that, due its low cost, minimum operational time, accuracy and versatility, the proposed application could constitute an interesting alternative method for quantitative studies of marine benthos.

2. Material and methods

Sampling was carried out on the rocky shore of the Pemaquid Point, Maine, USA, in July 2002. Benthic intertidal samples were obtained using 40 quadrats of 10×5 cm, randomly selected along a 5-m transect. Abundance was recorded as species cover (%). The cover of benthic invertebrates was estimated first by a visual scan method using a grid (Dethier et al., 1993), secondly by photographing the quadrat area and determining cover in the laboratory using a grid (photo quadrat), and thirdly by photographing the quadrat area and determining cover by means of digital image analysis.

2.1. Visual scan (VS)

Visual scan sampling was done using a grid of 32 small squares (1.25×1.25 cm each) superimposed on the quadrat frame (5×10 cm). Each square filled by a species counted as 3.25% cover, a square half filled count for 1.6% cover and organism filling less than 1/4 square counted for 0.5%. This method eliminates the need for decision rules such as any square>half filled is counted as filled (Dethier et al., 1993). The small size of quadrats (5×10 cm) allowed determining the percent cover under each small square.

2.2. Photo quadrat (PQ)

Photographs were also taken in the same area after removal of the grid used for the visual scan. One photo per 10×15 cm quadrat was taken using a digital camera (Nikon coolpix 990 at 3.1 megapixels). The camera was held directly over the target area perpendicularly attached to the quadrat frame using a 30-cm long rod, thus minimizing possible parallax errors. Photo analysis was performed following standard procedures of image analysis. Photographs were projected on the computer screen, then a computer grid, similar to the one used in the visual scan method (32 small squares of 1.25×1.25 cm each), was superimposed onto the photography. Computer grid was created using the Sigmascan Pro 5 software (SPSS science 1999). Cover was estimated using the same procedure rules as the ones used in VS.

2.3. Digital image analysis (DIA)

A second series of photographs was obtained for the same quadrats, using the same digital camera. At this time, photographs were analysed following digital image analysis procedures. Photographs (1024×768 pixel size) were transferred directly from the camera to the computer using JPG format. They were then converted into PIX format to be treated with the PCI software (PCI geomatics, 2000). PIX format is fully supported by GDB (generic database) libraries that allow different file types to be used interchangeably without loss of the radiometric resolution in the original image. Photographs in PIX format were then separated into RGB (red, green, blue) components (images). The images were first displayed in a RGB colour mode and radiometrically enhanced (linear method) to better display the information stored in their RGB components. Secondly, supervised, non-supervised classification, and high pass filter procedures were separately applied to all RGB images to determine the most accurate DIA method for cover estimation.

Image classification methods automatically categorize all pixels in an image into information classes and replace visual image with quantified information data (thematic maps). Supervised classification is based on the multispectral information contained in the image, labelling the pixels as members of a particular ground cover type or class (Richards, 1986). This procedure is made possible by some prior or acquired knowledge of specific sites in the scene that represent homogeneous examples of the known classes present in the scene. These areas are referred as training sites. Multivariate statistical parameters are then calculated for each training site with the goal of estimating the spectral characteristics or signatures for the classes present in the scene. Once these signatures have been determined, every pixel in the scene is evaluated and assigned to the class for which it has the maximum likelihood of being a member. This last step is done using appropriate classification algorithms.

Non-supervised classification (NSCL) on the other hand is a clustering procedure, partitioning the image into a number of spectral classes, totally unrelated to ground cover types (Glasbey and Horgan, 1995). Therefore, it does not start with a pre-determined set of classes as in a supervised classification but it requires an estimation of the number of groups present in the image.

A high band pass filter (Prewitt filter, in this paper) is part of a group of techniques applied directly to the image to increase its geometric detail (Richards, 1986). High pass filters emphasize abrupt changes in grey values between pixels, thus permitting the recognition of regions in the image where high spatial frequencies information (edges) is present. The filter procedure segments the image into groups but does not calculate automatically the number of pixels inside each group. Filtered images were therefore reconverted to JPG format for cover estimation using the Sigmascan Pro 5 software. Cover estimation was interactively determined by counting the number of pixels under the surfaces representing the organism under study. Percent cover for each species was determined by comparing the total number of pixels for each species to the total number of pixels in the image (total number of pixels for each species × total number of pixels in the image × 100^{-1}).

An ANOVA for repeated measures was performed to test for differences between the cover estimations obtained from the different sampling methods used in this study. Assumptions of normality and homoscedasticity were tested and data were transformed when necessary. A Tukey test for multiple comparisons was used when significant differences were observed. Finally, with the purpose of exploring the usefulness of the application of the estimation methods over a large set of data ($n = 100$, 500, 1000 quadrats), a Monte Carlo simulation analysis, based on the distribution parameters (i.e. mean and SD) of the real calculated data, was used. Again ANOVA for repeated measures was used to test for differences among methods when the number of sampling quadrats is increased.

3. Results

The visual census allowed to identify three benthic species whose presence was confirmed by the analysis of photographs and digital analysis of images. The species were, in decreasing order of abundance, *Semibalanus balanoides*, *Littorina littorea*, and *Mytilus edulis*.

Percent cover estimation was in general a time consuming task. The total operational time required in the field to estimate cover varied from 2 to 5 min per quadrat (Fig. 1). The use of a digital camera reduced this time to only a few seconds (30 s approximately) per image, the rest of the time being spent in the laboratory performing DIA. Besides, visual inspection of the digital data shows that 15 out of 40 (37%) images contained objects whose boundaries were not well defined. This lack of geometric definition limited proper species identification, leading to misclassification and therefore inaccurate estimations of percent cover. Similar spectral signatures, lack of contrast between the substratum and the object, and the small size of some of the individuals were responsible for this situation. The main goal of our DIA treatment was precisely to find a way to minimize these problems.

The digital camera used in this study offered the possibility of obtaining colour composite images (red, green, and blue components). Thus a multispectral analysis (e.g. supervised classification) based on these three components can be carried out. To determine the usefulness of such an approach, a correlation analysis was performed between the RGB components of the image. This analysis showed that pixel distributions on each component (RGB) were highly correlated (Table 1).

Supervised classification methods utilize the RGB multispectral information for differentiating the organisms present in the data. The high correlations shown between RGB components in our data imply a lack of sufficient spectral information to perform this task. Furthermore, as shown in Fig. 2A, supervised classification only detects two

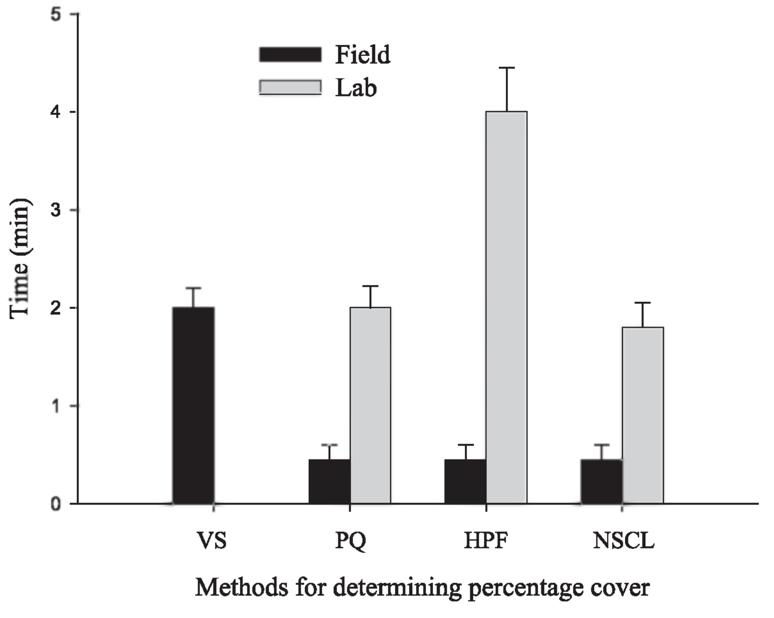

Fig. 1. Mean time used in each sampling and determination method. Mean is showed ± 1 S.D. VS, visual scan; PQ, photo quadrat; HPF, high pass filter; NSCL, non-supervised classification.

spectral groups, one of which corresponds to the zones of high concentration of encrusting algae. This is an inconvenience whose solution requires further studies beyond the scope of this work.

The non-supervised classification (NSCL) algorithm–Isodata–was used to determine how many groups could be recognized in the RGB data. Previous ANOVA test showed that the results from NSCL, applied in the RGB separately, do not show significant differences ($F=5.32$; $p<0.05$). Given this condition and the high correlation shown by the RGB components (Table 1), the red band was arbitrarily chosen to pursue the analysis. Fig. 2B presents a red image segmented by Isodata into four groups (see scale in Fig. 2B). Isodata identified correctly the organisms under study but also illustrates several potential problems. For example, *S. balanoides* was a mixture of groups three and four, *L. littorea*

Table 1
Mean values of the correlation coefficients between pixel distribution of the red, the green, and the blue components of digital image

		Digital image		
		R	G	B
Digital image	R	1		
	G	0.99	1	
	B	0.97	0.99	1

The correlation coefficients were obtained by simple linear regression using all pixels (1024 × 764) contained in each of the 40 images.

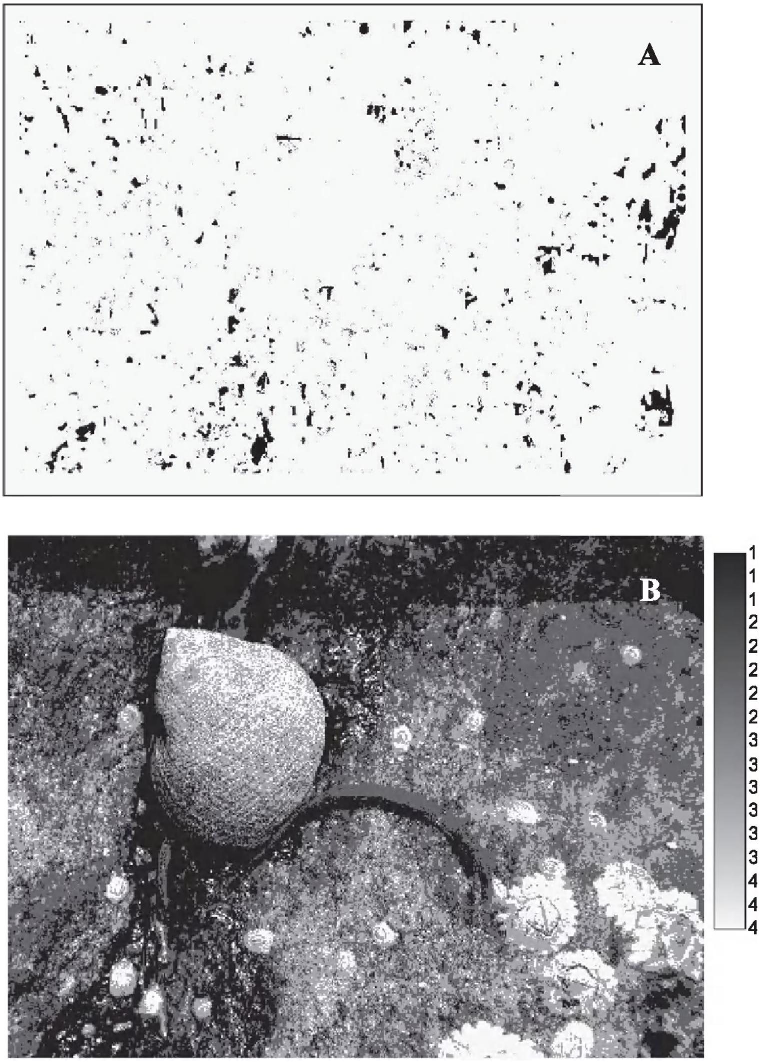

Fig. 2. Example of an image results obtained by (A) a supervised classification, and (B) a non-supervised classification of the same image. Fig. 2A shows two spectral classes, the black one represents zones with high concentration of encrusting algae. Four statistically different groups were found using supervised classification. Scale numbers, on the right of Fig. 2B, indicate the relation between the four groups and the corresponding gray values in the image.

included groups two and four, and these groups were also present on the bare rock substrate. In other words, organisms were subdivided into subgroups and there were also spectral overlaps; the high spatial resolution and the large dynamic range present in the data together with the incapability of the RGB bands to resolve the spectral characteristics of the groups were responsible for this situation. In general, non-supervised classification does not improve the recognition of individuals that in the original image were not well defined. Therefore, classification criteria based only on the grey value of the pixel, as Isodata, led to overlap of different groups and to inaccurate percent cover estimation (>50%).

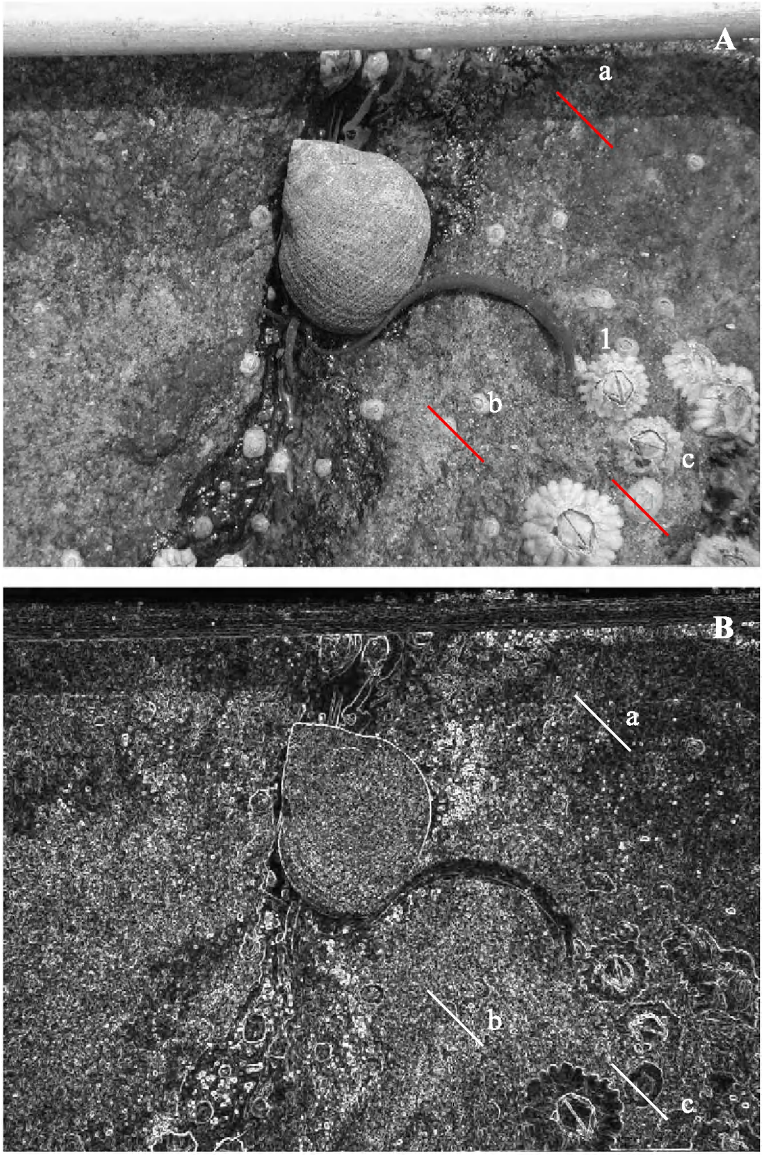

Fig. 3. An original image and its high pass filtered version. (A) Digital red band before application of the Prewitt filter, (B) after application of high pass filter. The lines (a, b, c) indicate segments analyzed for profile information. All segments are oriented in the same direction (left to right).

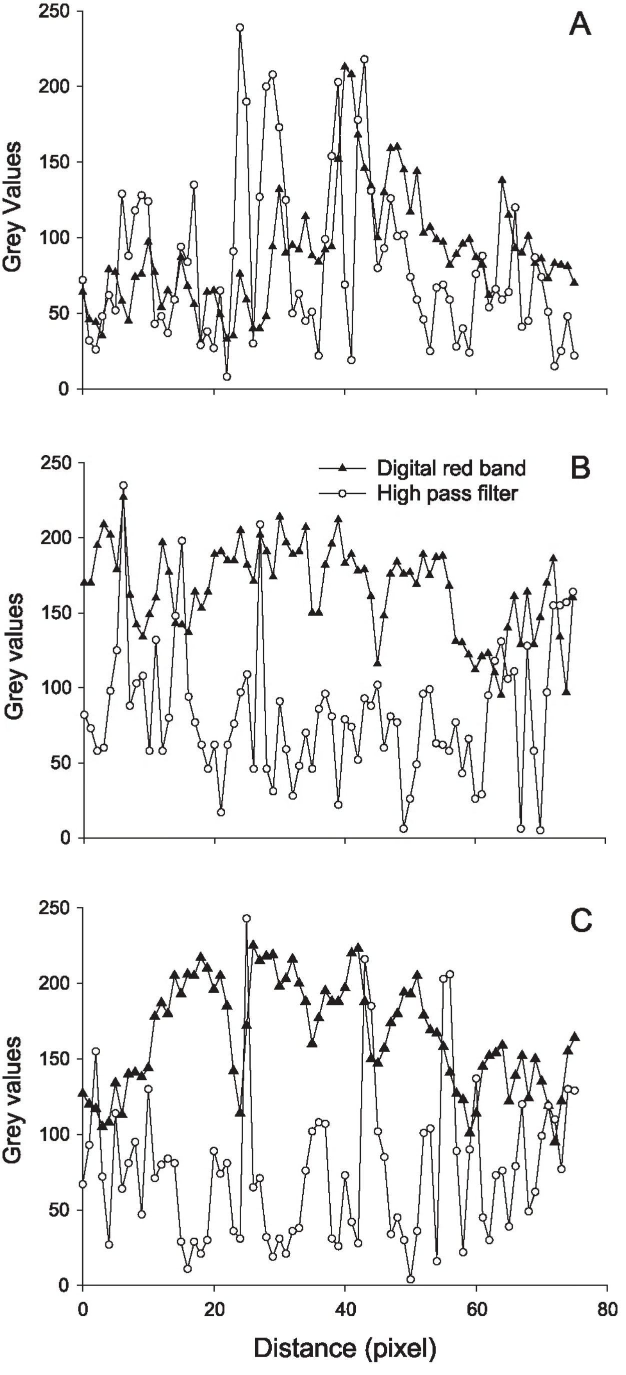

Fig. 4. Gray value profiles from the three sections shown in Fig. 3 of: (A) rock with noise (segment a in Fig. 3); (B) diffuse organism (segment b in Fig. 3); and (C) well-defined organism (segment c in Fig. 3).

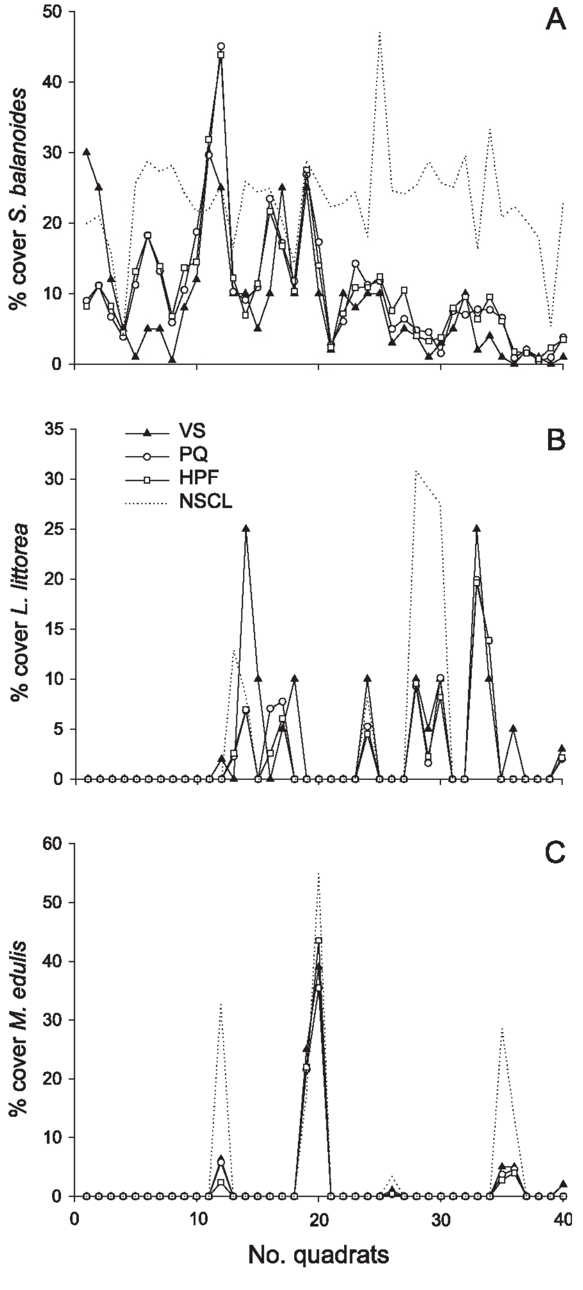

Fig. 5. Comparison of percent cover estimation values of (A) *S. balanoides*, (B) *L. littorea*, and (C) *M. edulis* obtained by different estimation methods used in this work: VS, visual scan; PQ, photo quadrat; HPF, high pass filter; NSCL, non-supervised classification.

Next, the original image was filtered using the high pass filter (see Fig. 3). To make the comparison meaningful, the same enhancement look-up-table was applied to both images. The filtered images emphasized the regions in the image where abrupt changes in grey values took place. Fig. 3B emphasizes the high frequency information, offering a greater contrast than the raw image. The discrimination between the image background and the benthic organisms is increased. To show this point more clearly, profiles, each 85 pixels long, were obtained in three different sections of the images (white lines in Fig. 3). The first segment (a) corresponded to a region where species were not present, the second one (b) was from a region where species were not well defined, and finally the third segment (c) was obtained from a region where a well defined species (*S. balanoides*) was present. All segments were oriented in the same direction (left to right).

These profiles, presented in Fig. 4, clearly indicate that in a region lacking the presence of species (Fig. 4A), there is no clear gain by filtering the original image because the profiles are basically superimposed on each other. However, where species are present (Fig. 4B and 4C), the filtered image offered a greater definition of such objects. For example, in Fig. 4C, the boundaries defining *S. balanoides* were well defined by a series of five maximum peaks situated in the middle of the graph. A similar pattern of five peaks, with similar numerical values, was also observed at the beginning of Fig. 4B, allowing, by analogy, to infer that the ill defined species in Fig. 4B were indeed of the same type (*S. balanoides*) than the one observed in Fig. 4C.

We have therefore four methods for estimating percent cover: the visual scan (VS), the analogue photo quadrat (PQ), the DIA using a non-supervised classification (NSCL) and the high pass filter (HPF). Comparison of these methods is presented in Fig. 5. DIA using HPF and the VS and PQ methods offered comparable estimates. However, the NSCL method diverged for all three species examined. Statistically, ANOVA for repeated measures test showed that indeed there were significant differences between the estimation methods (Table 2). For example, for *S. balanoides*, the estimation by NSCL method was higher than the other three for 80% of the cases (Fig. 5A). A similar situation was observed for *L. littorea* (Fig. 5B) and for *M. edulis* (Fig. 5C). According to the Tukey multiple

Table 2
Results from ANOVA for repeated measures to test for the differences between methods for determining percentage cover

	SS	MS	F	p	P(α)	Tukey (MCT)
S. balanoides	13165.44	4388.48	345.21	0.00	1.00	VS, PQ, HPF≠NSCL
Error	1983.16	12.71				
Total	15148.6					
L. littorea	474.33	144.91	4.07	0.01	0.81	VS, PQ, HPF≠NSCL
Error	1992.28	33.58				
Total	2427.56					
M. edulis	597.01	199.00	7.9	0.5	0.45	VS, PQ, HPF≠NSCL
Error	6088.36	253.34				
Total	6677.38					

VS = visual scan; PQ = photo quadrat, HPF, = high pass filter; NSCL = non-supervised classification. $p < 0.05$; P = power test value, $\alpha = 0.05$.

Table 3
Results from ANOVA for repeated measures using data from Monte Carlo simulation analysis

Species	*N*	SS	MS	*F*	*P*	P(α)	Tukey (MCT)
S. balanoides	100	438.38	219.19	2.44	0.089	1	
Error		26 719.39	89.96				
Total		27 157.78					
	500	1312.37	656.18	8.91	0.000	1	VS≠PQ, HPF
Error		110 240.4	73.64				
Total		111 552.8					
	1000	1694.17	847.07	11.57	0.000	1	VS≠PQ, HPF
Error		219 511.3	73.24				
Total		22 120.51					
L. littorea	100	707.86	353.93	10.25	0.000	0.98	VS≠PQ, HPF
Error		10 256.5	34.53				
Total		10 964.36					
	500	3509.46	1754.72	43.28	0.000	1.00	VS≠PQ, HPF
Error		60 697.86	40.54				
Total		64 207.32					
	1000	6897.44	3448.72	86.55	0.000	1.00	VS≠PQ, HPF
Error		119 422.82	39.84				
Total		126 320.21					
M. edulis	100	1059.01	529.5	2.75	0.065	0.54	
Error		57 167.63	192.48				
Total		58 226.64					
	500	3068.18	1534.09	8.41	0.000	0.80	PQ≠HPF
Error		273 216.53	182.5				
Total		276 284.72					
	1000	2191.95	1095.97	5.97	0.000	0.96	PQ≠HPF
Error		556 234.41	183.59				
Total		552 426.44					

Simulated data were calculated based on the mean and SD of the real calculated percent cover for each one of three species. VS = visual scan; PQ = photo quadrat; HPF = high pass filter. $p < 0.05$; P = power test value, $\alpha = 0.05$.

comparison test, the NSCL method exhibited significant differences in percent cover determination with respect to the other three methods. VS, PQ, and DIA using HPF do not present significant differences in their estimations.

Therefore, a Monte Carlo simulation test was only applied to VS, PQ, and DIA using HPF. Significant differences between them for percent cover estimation of each species were observed when the number of sampled quadrats was increased (Table 3). For example, *S. balanoides* and *L. littorea* cover estimations by the VS method differed from the PQ and DIA methods. For *M. edulis*, Tukey test detected only a significant difference between the PQ and DIA using HPF methods.

4. Discussion

Given the small size of quadrats used in this study (5 × 10 cm), the estimation of the number of taxa by the three methods was carried out without difficulty. Our calculations of percent cover using 40 quadrats, excluding the results obtained by NSCL, show no significant differences between the VS, PQ, and DIA using HPF. In the case of these last three methods, Monte Carlo simulations show significant differences when the number of quadrats is increased (>100 quadrats). This result is attributable to the fact that in both VS and PQ methods there are always a large number of unidentified or misclassified objects present. There is also a potential for misidentification of species whenever photographs or images are used, due to the fact that species with similar morphologies cannot be easily discriminated. Foster et al. (1991) have shown that photo quadrats consistently underestimate organisms cover and the number of taxa present. Using DIA by HPF, the estimation of organisms cover is a function of the number of pixels defining each object and, in turn, each object is defined by its boundaries in the filtered image.

The combination of a high-resolution digital camera as a data acquisition device and DIA techniques for the treatment of such data present several advantages and could constitute powerful tools for the study of benthic communities. The analysis of the biological information presented in this study shows that the results obtained using digital image analysis (DIA) by high pass filter (HPF) provided similar results to the ones already obtained by conventional methods (VS, PQ). However, it is not without potential problems. Table 4 lists some of the advantages and disadvantages in using these digital tools.

Probably one of the most important advantages of DIA is that using HPF is possible to increase resolution at the edge of the image, eliminating therefore the noise caused by wet surfaces and shadows. Another advantage is that the information extracted from the images is objective and quantitative, reducing in this way the variability produced by the effect of the observers (Dethier et al., 1993). It is well known that time is a critical factor in conducting experiments and observations in intertidal zone due mainly to the duration of the tidal cycle. Acquiring data using a digital camera is fast and flexible. This technology allows immediate revision of images, deletion and re-recording when necessary without having to wait for the developed films, as is the case with analogue cameras. Furthermore, digital images are directly saved either on memory cards or computer memories avoiding the use of digitizing procedures. Thus, 100% of the original high quality data is preserved in a short time and at minimum cost. Another point in favour of the use of high resolution camera in benthic studies is that images obtained by such cameras (in our case Nikon coolpix 990) with at least a resolution of 3.3 megapixels can be enlarged up to four times without loosing details. This property provides high frequency information on the organisms under study.

Table 4
Advantages and disadvantages in using digital tools (high resolution digital photography and digital image analysis)

Advantages	Disadvantages
Objective, quantitative	Estimates only the canopy cover
Speed for acquiring field data	May require a long laboratory process
Cost-effective for repetitive sampling	Expensive for one time sampling
Camera easy to use	Data analysis requires specialized training
Compatible digital image format	Requires large memory space
Allows the exploration of alternative ways to extract information	Time consuming in the laboratory
Provides information of three spectral bands	Requires specialized software

Another advantage of HPF is that it is a technique that does not require additional input from the user (i.e. colour ranges). Filter techniques based on convolution calculations allow automatic association of changes in brightness with pixel position (Richards, 1986), permitting in this way to delimit information classes (groups of pixels that form categories of interest). In our case, the high resolution available in our images provided us with quality information about the morphology of species; these geometric forms are easily identified in a high band pass filtered image. However, species do not present necessarily well-defined spectral signatures in a RGB space. The series of steps needed for this process can be routinely implemented on any digital image analysis package by a series of programmable computer procedures. Using this programmable procedure, the 40 raw images presented in this study were filtered in 15 min reducing thus the time needed for such analysis by about 60%. However, no method is without potential errors or problems; HPF does not determine automatically the number of pixels associated to the information classes; so, cover need to be interactively determinated by delimiting the edge boundaries of the organism. Another problem is that HPF only offers, similarly to the photo quadrat method, a two-dimensional view permitting to estimate the canopy covers (see also Meese and Tomich, 1992). Additional steps such as removal of algae may be necessary to estimate the under canopy species cover.

Given the high spatial resolution of our data, the high pass filter technique constitutes the most accurate method known to us so far. Although there may be conditions where it might be a useful technique, automatic colour segmentation based on colour ranges cannot be used routinely for accurate species discrimination in images from benthic sites (Bernhardt and Griffing, 1999).

The use of a high pass filter is only one of a series of possible DIA techniques. For example, other techniques suitable for determining organisms cover could be colour segmentation or supervised and non-supervised classifications, but in our case, the enhancement of information at the edges presented by the filtered image permits a better, global identification of invertebrate species and thus a more accurate estimation of percent cover. But, on the other hand, using classification methods is possible to detect areas with high concentration of incrusting algae.

In short, this study shows that the use of high-resolution photography together with high filtering techniques is a simpler and less expensive alternative to classical methods for obtaining and analyzing benthic samples at small spatial scales (cm). The use of a digital camera for acquiring data reduces substantially the temporal variability introduced inevitably by the sampling acquisition process. Digital image analysis using high pass filters increase the discrimination between the image background and the organism allowing a most accurate cover determination. Then this digital tools could therefore substantially lower the cost of monitoring programs where a fast acquisition data rate is needed at any spatial scale and improve the estimates of the ecological properties of the community.

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