Application of landscape mosaics for the biological quality assessment of subtidal macroalgae communities using the CFR index.

Guinda, X. [(1) *], A. Gracia [(1)], A. Puente [(1)], J.A. Juanes [(1)], Y. Rzhanov [(2)], L.A. Mayer [(2)]

(1) *Institute of Environmental Hydraulics (IH Cantabria), Universidad de Cantabria, PCTCAN, C/Isabel Torres nº 15, 39011, Santander, SPAIN.*
(2) *Center for Coastal & Ocean Mapping - Joint Hydrographic Center, University of New Hampshire, Jere A. Chase Ocean Engineering Lab, 24 Colovos Road, Durham, NH 03824, USA.*

Abstract

The assessment of anthropogenic impacts on coastal waters is an important task to accomplish under the European Water Framework Directive (WFD 2000/60/EEC). Macroalgae are one of the biological quality elements that must be considered, but their assessment has been generally limited to intertidal areas due to the difficulties and costs associated with working in subtidal areas. In this work, the suitability of using landscape mosaicing techniques is analyzed for the application of the "Quality of Rocky Bottoms" index (CFR by its Spanish acronym) in subtidal areas. For this purpose, the sensitivity and accuracy of both the indicators that compose the CFR index (characteristic macroalgae coverage, fraction of opportunistics and characteristic macroalgae richness) and the index itself were tested against different sampling surfaces and validated through direct applications of the CFR carried out *in situ* by scuba divers. The study was carried out at three sites, located on the coast of Cantabria (N. Spain), covering a variety of environmental conditions (depth ranges and anthropogenic pressures). Underwater video transects of 5-20 m length were recorded by scuba divers and processed with specialized software to build continuous image mosaics of the assessment sites. Each mosaic was inserted into a Geographical Information System where all distinguishable macroalgae species were identified and their coverages were estimated. Replicated subsamples of different areas (0.25 m^2, 0.5 m^2, 1 m^2 and 2.5 m^2) were tested from each mosaic for the estimation of both the single indicators and the CFR index itself. Main results showed that larger subsample areas produced higher and more accurate CFR values, mainly related to higher richness values and to smaller variability within the replicates. Accordingly, the minimum sample size required to carry out this type of studies was estimated to be of 2.5 m^2, showing no significant differences with the total mosaics. At this spatial scale, the assessments of the CFR index using mosaics showed a significant correlation and an excellent agreement with the results obtained *in situ*. In summary, underwater video mosaicing techniques proved to be a useful tool for the application of the CFR index and could also be of great interest for the study of subtidal environments by allowing visualization of extensive seafloor areas.

Keywords: Landscape mosaics, CFR index, subtidal macroalgae, sample size, Water Framework Directive.

*Corresponding author. Tel.: + 942 20 16 16 (Ext. 1118); fax: +34 942 26 63 61.
E-mail address: guindax@unican.es (Xabier Guinda).

1. Introduction

During the last decade, the requirements established by the European Water Framework Directive (WFD 2000/60/EEC) have motivated the development of several biotic indices for the assessment of different biological quality elements (phytoplankton, macroalgae, angiosperms, benthic invertebrates and fishes). Regarding macroalgae communities, most European countries have limited their assessments to the intertidal fringe due to costs and difficulties associated with working in subtidal areas. In this sense, most of the developed indices have been focused on their application to intertidal areas (e.g., Ballesteros *et al*. 2007; Bermejo *et al*., 2012; Díez *et al*., 2012a; Neto *et al*., 2012; Orfanidis *et al*. 2003; Pinedo *et al*. 2007; Wells et al., 2007) but only a few of them are appropriate for subtidal areas (Derrien and Legal, 2010; Carpentier *et al*., 2011) or for both intertidal and subtidal areas (Juanes *et al*., 2008). Most of these indices require precise species identifications, which makes their application in subtidal areas difficult. However, the "Quality of Rocky Bottoms" index (CFR by its Spanish acronym) (Juanes *et al*., 2008; Guinda *et al*., in preparation) uses an easy to apply methodology that does not require very precise taxonomical identifications because it is based in the assessment of general coverages of large characteristic macroalgae and opportunistic species. This simplification of the assessment procedure makes fast application of the index possible, which is very practical for extensive monitoring works or for its application to subtidal areas.

Most of the studies carried out at subtidal rocky bottoms require *in situ* sampling works that are usually performed by scuba divers. These studies are very time-consuming because they require visual assessments at various sampling units or quantitative sample collection works. In the case of visual assessments, divers must be skilled in taxonomic identification and assessment procedures and quantitative sample collections are extremely time-consuming and limited to small sampling areas. These inconveniences reduce the number of sampling units and the total areas that can be covered at each dive. To facilitate these surveys, other sampling techniques, such as underwater photography and video, have been used as an alternative. Photo-transects techniques have been successfully used for the study of several aspects regarding benthic communities, such as their structure and dynamics (Garrabou et al., 2002), long-term temporal changes

(Kollmann and Stachowitsch, 2001), continuous changes along depth gradients (Smale, 2008), coral reefs recovery after hurricane impacts (Coles and Brown, 2007), algal beds ecological monitoring (Ducrotoy and Simpson, 2001) and general monitoring works (Van Rein *et al*., 2011). Video techniques have been applied by Norris *et al*. (1997) for the assessment of subtidal seagrasses or, combined with hydroacoustic techniques, for the seafloor substrate classification (Rooper and Zimmermann, 2007). Combinations of underwater imagery and hydroacoustic techniques, together with modelling and automated classification techniques, have been useful for the development of predictive habitat distribution maps (Ierodiaconou *et al*., 2011; Holmes *et al*., 2008), which are very valuable for the extensive management of subtidal areas.

In addition to the reduced scientific expertise needed for divers and the reduced diving times needed for video recordings or photographs, these techniques provide the added benefit of permanent visual records, which can be later analyzed in the laboratory, looking for additional information in the images. However, one of the main weaknesses of the photography and video surveys is their low resolution; species must be identified from a photograph or from individual video frames, which can be difficult in case of small-sized organisms. This limitation is partially compensated by the possibility of having information from large surveyed areas, which are especially attractive for extensive assessments or monitoring studies. A step forward in this sense has been achieved with the development of video and photo mosaicing techniques (e.g. Gracias and Santos-Victor, 1998; Marks *et al*., 1995; Rzhanov *et al*., 2000; Rzhanov *et al*., 2007) that allow the creation of large images of the seafloor by mosaicing several photographs or video frames, thus providing a wide vision of the structure and composition of benthic assemblages in the surveyed area. In this aspect, Parravicini *et al*. (2009) and Kaiser (2003) considered that sampling unit size, rather than sampling method, is the crucial factor to take into account in sampling design. Consequently, it is necessary to define, according to the pursued objectives, the minimum sampling area required for each type of study. Most studies of subtidal environments use small sampling quadrats that generally range between 0.025 and 1 m^2 (e.g. Carpentier *et al*., 2011; Alvaro *et al*., 2008; Garrabou *et al*., 2002; Parravicini *et al*., 2010). In contrast, video mosaic analyses are based on records of wide areas that can range between 10 and 600 m^2 (e.g. Lirman *et al*., 2007; Lirman *et al*., 2010; Ludvigsen *et al*., 2007) and subsampling of different quadrats with areas between 0.25 and 1 m^2 (Lirman *et al*., 2007; Lirman *et al*., 2010). Video-mosaicing techniques have been used in different types of underwater studies, such as the assessment of coral reefs status (Lirman *et al*., 2007), recovery of reefs after injuries suffered by vessel groundings (Lirman *et al*., 2010) and by hurricane impacts (Gleason *et al*., 2007). Besides biological applications, these techniques have been also used in deep-sea archaeological surveys (Søreide and Jasinski, 2005; Ludvigsen *et al*., 2007). Geographic Information Systems (GIS) provide a very useful tool for these applications, as they allow carrying out spatial analyses (e.g. estimation of coverage percentages of different biological species) over large geo-referenced videomosaics (Jerosch *et al*. 2006).

The use of Remotely Operated Vehicles (ROVs) and underwater towed cameras has provided an additional tool to survey deep subtidal areas (e.g Guinan *et al*., 2009; Lorance *et al*., 2006; Norcross *et al*., 1999; Rzhanov *et al*., 2007). These systems reduce the inherent limitations of scuba divers because they can reach deeper depths and provide longer underwater time, thereby increasing the possibility of carrying out more extensive surveys at greater depth ranges. In shallow areas, the use of ROVs can be also very useful as they allow surveying a great number of sampling stations in the same day, which is not possible by scuba diving, thus reducing the temporal variability and costs of the surveys. This advantage can be even more interesting in highly hydrodynamic coastal regions, such as the Cantabrian Sea (Castanedo *et al*., 2006; Valencia *et al*., 2004), where the number of subtidal surveying available days can be very limited.

Finally, the use of non-destructive sampling methods, included in the recommendations of the International Council for the Exploration of the Sea (ICES, 2001), assumes less environmental damage and absence of laboratory work, thereby simplifying data processing and notably reducing the total monitoring costs (Ballesteros *et al*., 2007; DEFRA, 2004; García-Castrillo *et al*., 2000). Non-destructive sampling methods in underwater surveys require fast visual assessments that usually cannot allow for detailed taxonomical identifications. In this sense, the level of taxonomic detail required in the studies should be taken into account based on the pursued objectives. Since Ellis (1985) introduced the concept of taxonomic sufficiency, many studies have demonstrated that, in some cases, identification of organisms to higher taxonomic levels, such as family or order, is sufficient to achieve the desired objectives (Díez *et al*., 2010; Ferraro and Cole, 1990; Puente and Juanes, 2008; Somerfield and Clarke, 1995; Warwick, 1988a, 1988b).

The assessment of the CFR index is based in an ecological approach that does not require a precise taxonomical identification of macroalgae species and which application should be carried out over extensive survey areas. Hence, considering all the above mentioned aspects, the use of underwater videomosaics as large subtidal sampling units, combined with the use of GIS applications for the identification and quantification of main macroalgae species, and the application of the CFR index (Juanes *et al*., 2008), might be a low-cost and effective strategy for the rapid assessment of subtidal macroalgae assemblages in order to carry out extensive management or monitoring works. In this sense, one of the main aspects that should be solved is the minimum sampling area required for accurate and reliable results.

According to these guidelines, the aim of this work is to assess the suitability of using seafloor video mosaics for the application of the CFR index in subtidal areas. For this purpose, two specific objectives are established: i) to analyze the sensitivity and accuracy of the estimation of both the indicators that compose the CFR index (characteristic macroalgae coverage, fraction of opportunistics and characteristic macroalgae richness) and the index itself using different sampling surfaces and ii) to validate the obtained results through direct applications of the CFR index in the field.

2. Material and Methods

2.1. Study Area

The study was carried out in the summer of 2011 at three sites located on the coast of Cantabria (N. Spain) (Figure 1). At the first site, Castro (CA), an urban effluent is discharged through a submarine outfall. The second place, Ontón (ON), is located near the industrial effluent of a fluoride factory that discharges directly to the intertidal zone. The third site, Callejos de Bamboa (CB), is located between Castro and Ontón. Three sampling stations were established in Castro and Ontón and two were established in Callejos de Bamboa. The stations were classified according to three depth ranges; shallow (S), between 10-15 m depth, was only represented by ON-1 station, medium

(M), between 15-20 m, was represented by CA-1, CB-1 and ON-2, and deep (D), between 20-25 m, was represented by CA-2, CA-3, CB-2 and ON-3.

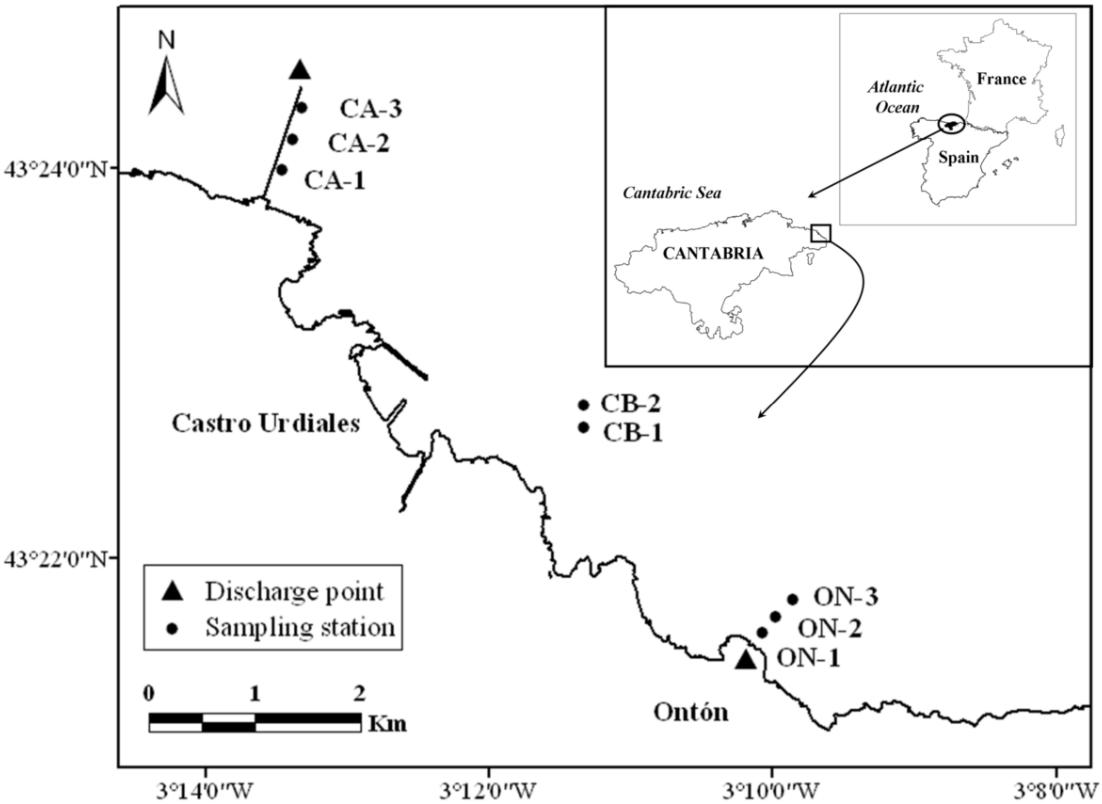

Figure 1. Location of the sampling stations and the discharge points at each studied site on the coast of Cantabria (N. Spain).

2.2. Survey methodology

At each station, two to three transects of 5 to 20 m length and 1 m wide were video recorded by scuba divers over stable rocky substrates that were suitable for macroalgae colonization. At the beginning of each transect, a 50 x 50 cm square was set to provide a real scale of the final mosaics. In addition, the CFR index (Juanes *et al*., 2008) was visually applied *in situ* by skilled divers over the whole area of each transect, according to the last version accepted in the European Intercalibration process for the Water Framework Directive implementation (Guinda *et al*., unpublished; JRC, 2011).

2.3. Mosaic construction

In order to create landscape video-mosaics from the recorded transects, the number of frames of the underwater videos were reduced to get an overlap between consecutive frames about 80% (1.5-3 frames per second approximately), thus reducing the time required for the mosaic construction process. The mosaic construction programs used were developed at the Center for Coastal and Ocean Mapping (CCOM) of the University of New Hampshire (Rzhanov *et a*l., 2000). The process comprises four major stages that were run iteratively to get the most visually consistent results (Figure 2). The first stage established common linking points between successive pairs of frames using the *Feature Co-Reg* application. As a result, an output RRA file was generated with the registration parameters. Sometimes, some failures in finding relative homography occurred; in those cases, linking points were manually chosen between different pairs of frames using the *Feature* application. The information about these corrected linking points was saved in the original RRA file. Moreover, the tilt, pitch and roll of the camera were compensated in the mosaic-building process by the *Tilt Correct* application. The fourth stage created the video mosaic of the imaged area using the *Build Mosaic* application. If the obtained mosaic was not satisfactory, then the process was iteratively repeated until a satisfactory mosaic was obtained (Figure 3A).

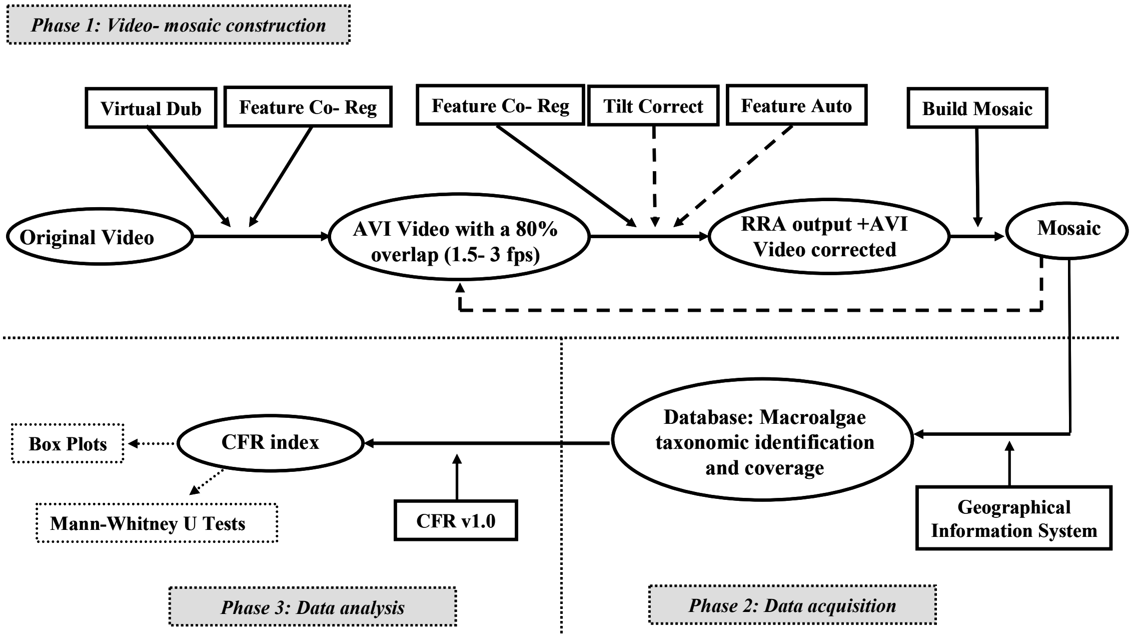

Figure 2. Stages of the methodology followed for the application of the CFR index using video-mosaics. Dashed lines represent processes which are not mandatory for the process.

2.4. Data acquisition

Seafloor video-mosaic images were geo-referenced, incorporated into a Geographical Information System (ArcGis v10.0) and adjusted to the real size, as it is shown in Figure 3A. At each mosaic, all distinguishable macroalgae species were identified and outlined, which produced polygons whose areas were measured and registered (Figure 3B). This information was extracted for the whole mosaics and for several subsamples of four different sizes (0.25 m^2, 0.5 m^2, 1 m^2 and 2.5 m^2). Depending on the number of transects per station (two for deeper ones and three for the shallower) and their lengths, the number of subsamples per station ranged between six to nine replicates for the smaller sized subsamples (0.25-1 m^2), and between five to six replicates for the 2.5 m^2 subsamples. The subsamples were equidistantly set along each mosaic (Figure 3C).

The taxonomic identification was carried out to the lowest possible level. However, when macroalgae could not be identified to the species level, groups of species and mixed communities were also established. Thus, delicately branched red filamentous species with a noticeable size, such as *Heterosiphonia plumosa*, *Bornetia secundiflora* and some species of the genera Polysiphonia and Pteroshiphonia, were classified as “red filamentous algae”, whereas mixed communities of small-sized algae were identified as “low bearing algae”. All of these species are equally considered in the CFR index; consequently, the missing taxonomical precision in this case does not affect the final results. In the case of Ontón, a high abundance of an epiphytic red filamentous algae mat was found covering other algae and most parts of the substrate; hence, it was classified as “red opportunistic filamentous” (ROF) due to its high proliferation. A small sample of the algae mat was taken to the laboratory and identified as a combination of species dominated by *Trailliella intricata* and *Falkenbergia rufolanosa*.

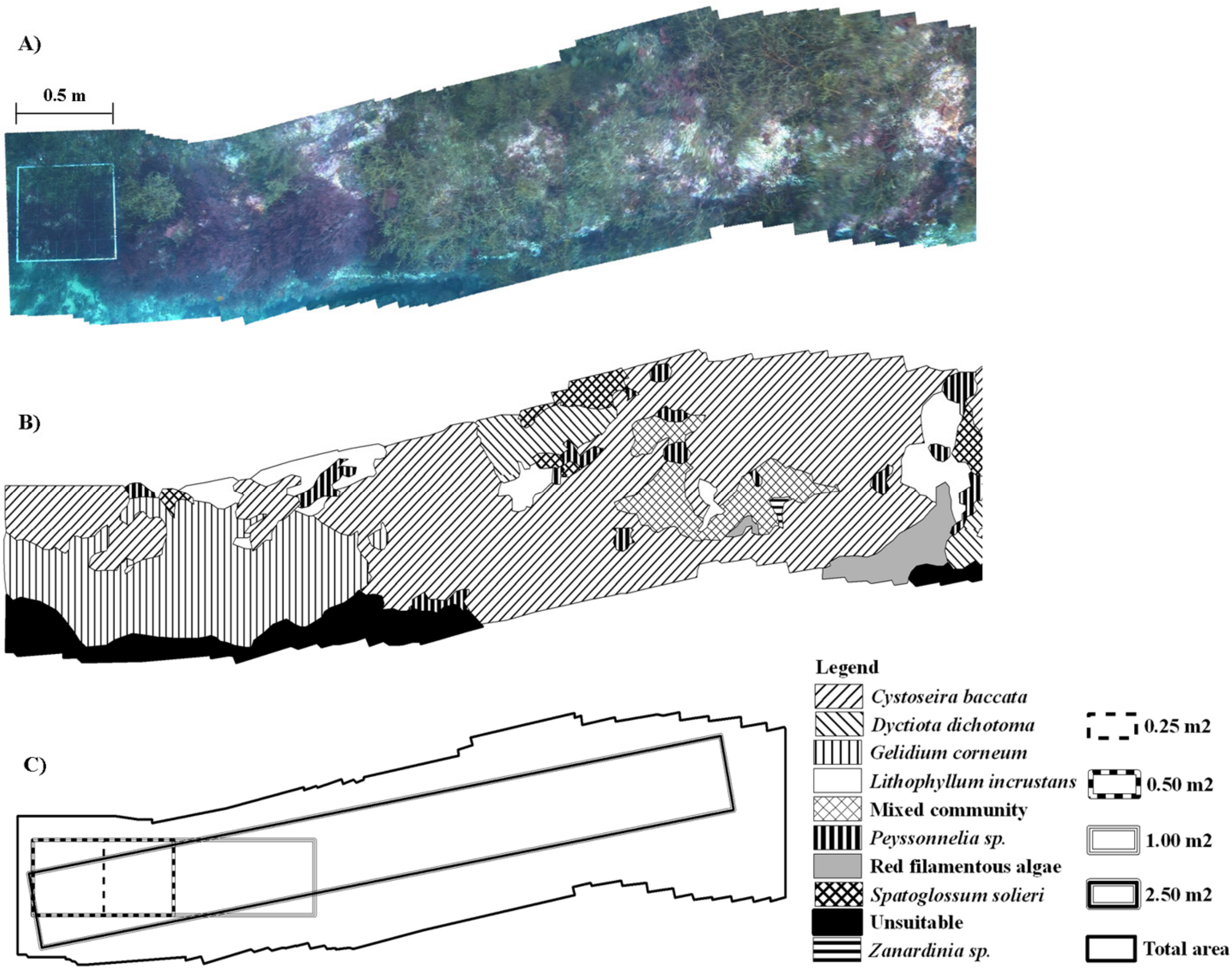

Figure 3. A) Fragment (first third) of a surveyed transect with a reference square at the beginning of the mosaic. B) Taxonomical identification of different macroalgae species using GIS. C) Subsamples of different sizes performed at the beginning of the landscape mosaic.

2.5. Data analysis

Using the coverage values of the identified macroalgae species, the individual indicators that comprise the CFR index were calculated (characteristic macroalgae coverage (C), fraction of opportunistic species (F) and characteristic macroalgae richness (R)) and the CFR index was applied following the same methodological approach mentioned in the section 2.2. (Juanes *et al.*, 2008; Guinda *et al.*, unpublished; JRC, 2011) (Figure 2). The calculations were carried out for the different subsamples and for the total mosaics. Shadows, fauna or substrates that are unsuitable for macroalgae colonization, such as sediments or small stones, were not considered in the analyses; consequently, these areas were disregarded in the analyses.

In order to analyze the existence of significant differences in the results obtained using different subsample areas and the results obtained using the whole mosaics, Mann-Whitney U Tests were applied by pairs for each indicator and for the CFR index.

The results of the CFR index applied over the whole mosaics were finally compared with the results obtained *in situ* in order to validate the accuracy of the proposed methodology for the application of the CFR index. The comparison was carried out i) by the application of a linear regression between the CFR results obtained by the two procedures (visual and mosaics) and ii) by the application of a weighted kappa analysis (Monserud and Leemans, 1992) between the quality classifications obtained by the two procedures.

3. Results

Nineteen mosaics were constructed from the videos recorded at each of the three studied sites. Their total lengths ranged from 6.6 to 19 m (average of 13.7 m), which represent total surveyed areas between 4.9 and 21.1 m^2.

In general terms, the shallower stations were characterized by the presence of species such as *Cystoseira baccata*, *Gelidium corneum* and *Peyssonnelia squamaria*, while the deepest stations were characterized by the presence of *Halopteris filicina*. However, the higher coverages in all cases corresponded to a combination of low bearing algae, red filamentous algae and encrusting species. In the case of Ontón, high coverages of *Trailliella intricata* and *Falkenbergia rufolanosa* (ROF) were also detected. According to the information summarized in Table 1, *Cystoseira baccata* was the dominant characteristic macroalgae in the medium-depth stations of Castro (CA-1) and Callejos de Bamboa (CB-1), reaching an average coverage of 74.1% in Castro. The deepest stations of Castro (CA-2, CA-3) and Callejos de Bamboa (CB-2) had *Halopteris filicina* as the dominant characteristic macroalgae species, with coverages that reached 32.3% in Castro. At the deepest station of Ontón (ON-3) only low coverages of *H. filicina* were detected. *Calliblepharis ciliata* appeared only at mid depth in Ontón (ON-2), with a coverage of 8%, and *Codium tomentosum* and *Gelidium corneum* were found only at shallow and medium-depth stations with sparse coverage values (<5.6%). In addition, a low coverage of *Corallina* sp. was identified in the shallowest station of Ontón (ON-1). *Peyssonnelia squamaria* and *Spatoglossum solieri* were the most represented species, appearing in five stations that correspond to the three sites; however, their coverages were generally low, except in Callejos de Bamboa, where they reached maximum coverages of 6% and 4.9%, respectively.

Apart from the characteristic species, other accompanying species were also identified. In this case, encrusting macroalgae (*Lithophyllum incrustans* and/or *Mesophyllum lichenoides*), red filamentous algae and low bearing algae were the most conspicuous accompanying groups. Regarding opportunistic species, only Ontón showed important coverages. The general structure of the macroalgae community in all the stations surveyed in Ontón was composed mostly by low bearing algae and small patches of some characteristic species (e.g., *C. ciliata, G. Corneum, H. filicina, P. squamaria*), red encrusters (specially *M. lichenoides* in ON-1) and red filamentous algae. All of the above were mostly covered by a thin layer of *T. intricata* and *F. rufolanosa*.

Species/Station	Average Macroalgae Coverage per station (%)							
	CA-1	**CA-2**	**CA-3**	**CB-1**	**CB-2**	**ON-1**	**ON-2**	**ON-3**
Characteristic macroalgae:								
Calliblepharis ciliata (R)							8,02	
Codium tomentosum (G)	0,23			0,17		0,28		
Corallina sp. (R)						0,04		
Cystoseira baccata (B)	74,06	0,07		15,40				
Dyctiopteris membranacea (B)					0,16			
Dyctiota dichotoma (B)		7,41	3,05	7,95	12,93			
Gelidium corneum (R)	0,29			5,59		1,22		
Halopteris filicina (B)		24,49	32,33	0,31	9,88			1,90
Halydris siliquosa (B)	10,73				0,92			
Laminaria ochroleuca (B)	0,02							
Peyssonnelia squamaria (R)			0,05	5,97	0,35	1,48	0,46	
Spatoglossum solieri (B)	1,55	0,04		2,34	4,90		0,02	
Other macroalgae:								
Red filamentous algae (R)	4,96	10,28	29,35	1,20	17,30		0,50	2,84
Lithophyllum incrustans (R)	2,09	3,88	5,12	13,34	5,98		1,55	1,92
Mesophyllum lichenoides (R)		1,05				26,15		
Encrusting *Peyssonnelia* (R)	0,28				0,77			
Low bearing algae (Mixed)	2,90	27,70	26,27	44,77	22,94	32,51	44,59	46,56
Zanardinia sp. (B)				2,38	2,01	5,80		
Opportunistic species:								
Red Opportunistic Filamentous (R)						32,51	44,46	46,56

Table 1. Average macroalgae coverage per station corresponding to total transect surfaces (R: Red, B: Brown, G: Green species).

The results of the indicators that comprise the CFR index and the final CFR values obtained at each station are represented by Box plots in the Figure 4. The results include the values that correspond to the whole mosaic areas and to the different subsample sizes considered.

Characteristic macroalgae coverage (Figure 4A). The largest coverages of characteristic macroalgae, ranging from 85% to 95%, were observed at the medium depth station of Castro (CA-1), whereas the lowest coverages, ranging from 1.5% to 2.1%, were observed at the deepest station of Ontón (ON-3). At Castro and Callejos de Bamboa, the largest coverages were observed at medium depths (CA-1 and CB-1), with values of 86.9% and 37.7%, respectively, taking into account the whole mosaics areas. The deepest stations of Callejos de Bamboa and Castro (CB-2, CA-2 and CA-3) showed similar results, ranging from 29% to 35.4%, respectively. In Ontón, characteristic macroalgae coverage was highest (8.5%) at medium depth station (ON-2). The variability among subsamples is higher for the smallest areas (0.25 m^2) and it decreases with increased sample size. Whereas the average coverage values are not very different among different sample sizes, the results obtained among subsamples of the same mosaic vary enormously (e.g. between 10.6% and 100% for the 0.25 m^2 subsamples in CB-2). At Ontón, where coverage values are very small, these differences are not so high, and at ON-3 the coverage values range between 0% and 9.72% among the 0.25 m^2 subsamples. CA-1 constitutes a special case because the variability of the smallest subsamples is very small (90.7% to 100%), whereas the highest variability is found among the 1m^2 subsamples (58.6% to 99.2%).

Fraction of Opportunistic species (Figure 4B). At Ontón, high coverages of opportunistic species (30-50%) were found, which, considering the low characteristic macroalgae coverage values obtained in these stations, corresponded to average fraction of opportunistic values that range from 78.5% to 96.4%. The highest fractions of opportunistics are observed in ON-3, associated with the smaller characteristic macroalgae coverages. Just as with coverage values, the variability of the results is highest for the smaller sample areas (0.25m^2) and decrease with the increased sample size.

Characteristic macroalgae richness (Figure 4C). Considering the total area of the mosaics, the highest characteristic macroalgae richness values were found at CB-1, with 7 species, followed by CB-2 and CA-1, with 4 to 5 species. Deep stations from Castro (CA-2 and CA-3) obtained 3-4 species, slightly better results than ON-1 and ON-2 (2-3 species). Deep station from Ontón (ON-3) obtained the worst results, with only one species on average. In this case, the effect of sample size is very important, because bigger sample sizes obtained markedly higher richness values at all the stations. The variability among samples of the same size is not as high because the obtained richness values are low in all cases and negligible when compared to the differences observed among different sample sizes.

CFR index (Figure 4D). The results of the CFR index showed two clear groups of stations; a first group composed of Castro and Callejos de Bamboa stations and a second group that relates to Ontón stations. The first group, with average CFR values for the total area of the mosaics that range from 0.66 to 0.95, corresponds to the stations with the better qualities of macroalgae assemblages. According to the classification system established for the application of the CFR index under the Water Framework Directive (Juanes *et al*., 2008; Guinda *et al.*, unpublished; JRC, 2011), these values correspond to quality classes between “high” and “good”. The stations of the second group, with average CFR values below 0.15 in all cases, are classified as having a “bad” quality. In the three sites, deeper stations obtained lower CFR values, mainly associated to lower characteristic macroalgae coverages. Analyzing subsamples of different areas, bigger samples produced higher CFR values, associated with higher richness values, and more accurate results, because of smaller variability in the replicates. As a result, deeper stations from Castro (CA-2) and Callejos de Bamboa (CB-2) produced different quality classifications that depended on the size of the samples. In this case, smaller samples produced “moderate” qualities, whereas larger samples produced “good” qualities.

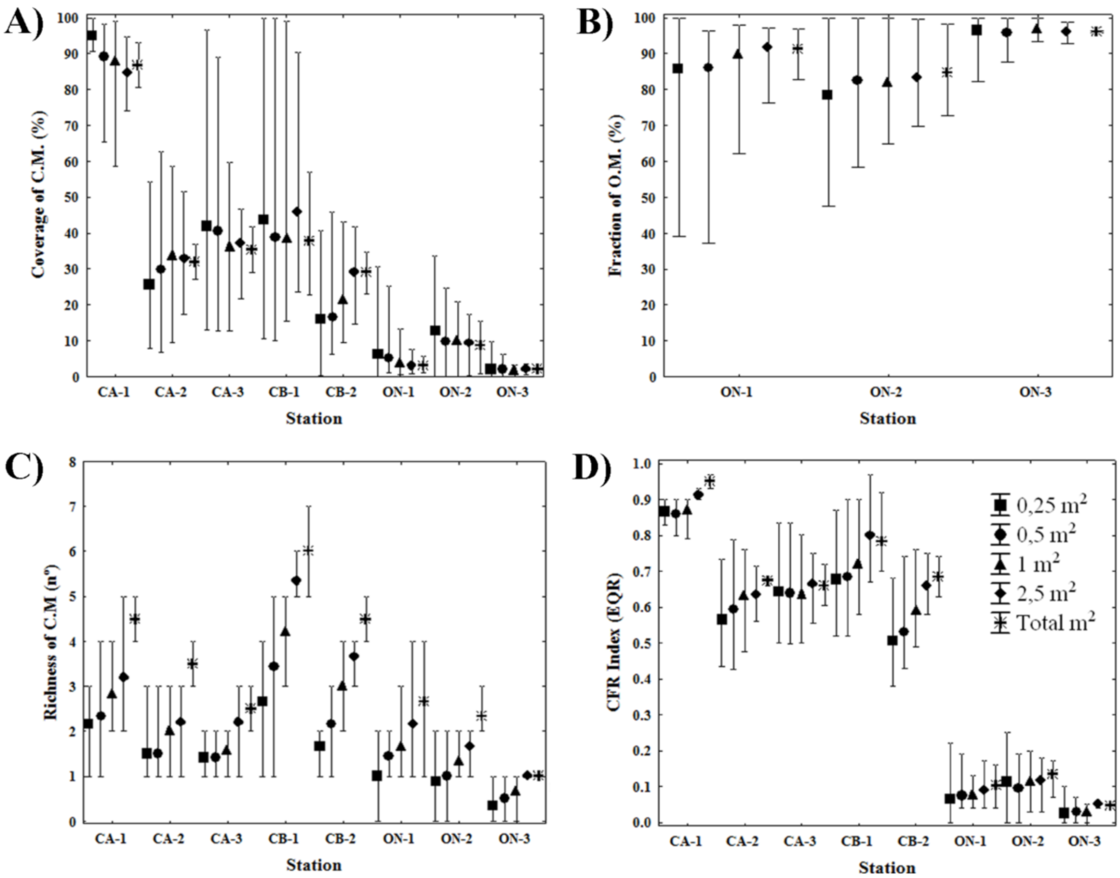

Figure 4. Box plots of the results obtained for the different indicators of the CFR index, with different subsample sizes. A) Characteristic macroalgae coverage, B) Fraction of opportunistic species, C) richness of characteristic species, D) CFR index.

The results of the Mann-Whitney U Tests applied by pairs, between subsamples of different sizes and the values of the total area of the mosaics, for each of the indicators of the CFR index, are shown in Table 3. Five stations (CA-1, CA-2, CB-1, CB-2, ON-2) show significant differences in richness values for one or more sample sizes with respect to the total areas. These differences are larger in the smallest sample sizes and decrease as sample size increases. Thus, in the case of 1 m^2 sample sizes, only two stations (CB-1 and ON-2) show significant differences with respect to the total area and, in the case of 2.5 m^2 samples, no significant differences are observed for any station. CFR index results are significantly different only for CA-1. These differences were detected even at 1 m^2 sample size, but they disappear at the 2.5 m^2 sample size. No significant differences have been observed in the coverages of characteristic macroalgae and in the fraction of opportunistics.

Station	Coverage of C.M.				Fraction of opportunistic species			
	0,25 m^2	**0,5 m^2**	**1 m^2**	**2,5 m^2**	**0,25 m^2**	**0,5 m^2**	**1 m^2**	**2,5 m^2**
CA-1	0,182	0,505	0,739	1,000				
CA-2	0,505	0,739	1,000	1,000				
CA-3	1,000	1,000	0,770	0,699				
CB-1	0,926	0,518	0,644	0,439				
CB-2	0,317	0,317	0,317	1,000				
ON-1	0,780	0,644	0,926	0,796	0,926	0,644	0,926	1,000
ON-2	0,926	0,926	0,644	0,796	0,926	0,926	0,644	0,796
ON-3	0,287	0,495	0,737	1,000	0,287	0,495	0,737	1,000

Station	Richness of C.M				CFR index			
	0,25 m^2	**0,5 m^2**	**1 m^2**	**2,5 m^2**	**0,25 m^2**	**0,5 m^2**	**1 m^2**	**2,5 m^2**
CA-1	**0,039***	0,062	0,080	0,232	**0,035***	**0,039***	**0,039***	0,094
CA-2	**0,049***	**0,049***	0,084	0,105	0,182	0,182	0,317	0,699
CA-3	0,078	0,078	0,102	0,676	0,769	0,769	0,769	0,699
CB-1	**0,010***	**0,018***	**0,019***	0,248	0,166	0,116	0,229	0,606
CB-2	**0,032***	**0,039***	0,049	0,124	0,096	0,182	0,317	0,739
ON-1	0,086	0,159	0,232	0,581	0,306	0,644	0,518	0,796
ON-2	**0,013***	**0,009***	**0,031***	0,123	0,781	0,518	0,518	0,796
ON-3	0,127	0,237	0,378	1,000	0,287	0,495	0,737	1,000

Table 3. Listing of the p values obtained in the Mann-Whitney U Tests by pairs, carried out among the results obtained for the indicators of the CFR index at different subsample sizes and the values corresponding to the total areas, for each station. * Significant differences ($p<0.05$).

As observed in Figure 5, the results of the CFR index obtained by visual applications *in situ* and the CFR results obtained over the mosaics were highly correlated (R^2=0.94; $p<0.01$). Regarding the assignation of quality classes, the percentage of agreement was 63.2% and the resulting weighted kappa value was 0.92, which corresponds to an “excellent” prediction level according to Monserud and Leemans (1992).

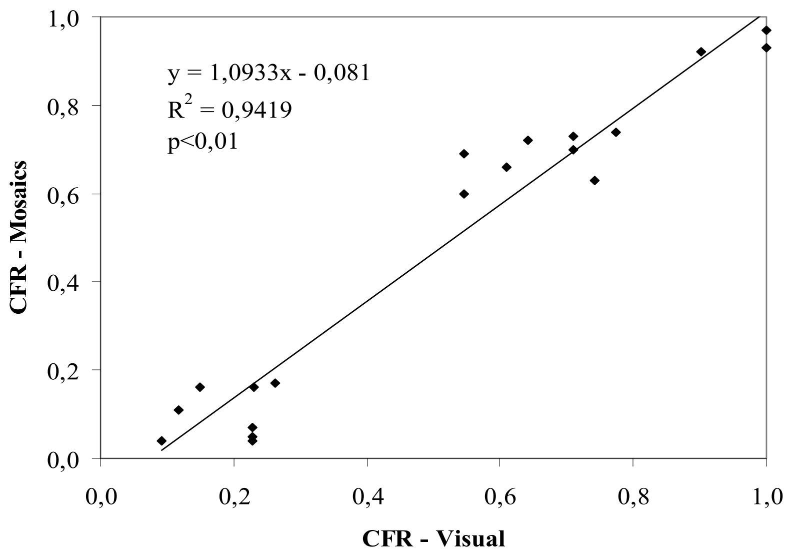

Figure 5. Correlation between the results obtained for the CFR index by direct *in situ* applications (X axis) and by indirect applications using mosaics (Y axis). According to the last version of the CFR index accepted in the European Intercalibration process for the Water Framework Directive implementation, the boundaries among quality classes are the following: High-Good: 0.81; Good-Moderate: 0.6; Moderate-Poor: 0.4, Poor-Bad: 0.2) (Guinda *et al*., unpublished; JRC, 2011).

4. Discussion

The results obtained in this work demonstrate the suitability of the landscape video-mosaicing techniques for the application of the CFR index in subtidal areas. This methodology allows obtaining accurate assessments of the composition of biological communities over extensive subtidal areas in a fast, easy and economical way, hence, it could be considered as an appropriate system for the monitoring and management of these environments.

As stated by Carpentier *et al*. (2011), a method for the assessment of subtidal macroalgae communities must be simple and rapid to perform. It must also allow divers, in some cases unskilled in algal taxonomy, to carry out reproducible and easy assessments and must synthesize the environmental data in order to advise managers on the decision-making process (Juanes *et al*., 2008). The proposed non-destructive method can be a useful technique for the extensive assessment and monitoring of macroalgae communities and to improve existing knowledge about subtidal communities by reducing to a great extent the costs associated to open-sea surveys. These reductions are achieved by increasing the number of sampling sites and the extension of the surveyed areas, while reducing the diving times and the number of sampling days. In addition, the reduction of sampling days may reduce also the temporal variability during the study period. These advantages can be very interesting in highly hydrodynamic coastal regions, such as the Cantabrian Sea (Castanedo *et al.*, 2006; Valencia *et al*., 2004) where the number of days available to carry out subtidal surveys may be very limited. Furthermore, mosaicing techniques can also be applied to videos recorded by Remotely Operated Vehicles (ROVs), thus extending its capacities to deep-sea research studies (Rzhanov *et al*., 2007) and reducing even more the number of sampling days required to cover a higher number of sampling stations, which is not possible by scuba diving. Other type of extensive studies that could be carried out with this technology might be associated with the quantification of marine resources (e.g. *Gelidium corneum*), or with the trend analyses of benthic communities, such as the Laminarians retreat in the Cantabrian region (Fernández, 2011; Díez *et al*., 2012b).

If we analyze the precision and accuracy of the obtained results, clear differences are found among the three indicators that compose the CFR index and, consequently, in the index itself. In the case of characteristic macroalgae coverage and fraction of opportunistics, the obtained results did not show significant differences for any of the sampling-unit sizes used, what indicates a good accuracy of the method in these cases. However, the results obtained with bigger sampling-unit sizes showed smaller variability in the results, thus increasing the precision of the assessments. On the other hand, richness and CFR index showed also a smaller variability when using bigger sampling-unit sizes (more precision), but the accuracy of the smaller samples was lower, what produced significant differences in some cases.

These effects can be explained by the heterogeneous "patchiness" of macroalgae aggregations, which requires large sampling areas or elevated number of samples to obtain a good representation of their real distribution. In this sense, the number of replicates used in this study for the estimation of characteristic macroalgae coverage and fraction of opportunistics was enough to obtain appropriate results with any of the tested sample surfaces. However, richness increases in direct relation to the variety of habitats sampled, therefore, it is obvious that higher richness values might be expected from larger sampling areas (Walther *et al*., 1995; Condit *et al*., 1996; Gotelli and Colwell, 2001). Thus, richness is nearly always underestimated in inventories because of its strong dependency on sampling effort (Melo *et al*., 2007). A long-standing question for ecologists has been to estimate the minimum sampling area required to obtain accurate richness values for a community (Evans *et al*., 1955; Keating *et al*., 1998; Melo *et al*., 2003). As it can be seen in the present study, the lower accuracy obtained with the smaller samples was mainly due to the underestimation of the richness values and the subsequent underestimation of the CFR index. In both cases, the accuracy and precision increased with the increasing sample sizes. In this sense, the Mann-Whitney test results (Table 3) indicated that the differences in richness values were not significant for any of the stations for sample areas larger than 2.5 m^2. Therefore, this sample area (2.5 m^2) could be considered as the minimum sample size recommended for this kind of study. It must be also considered that the resolution limitations of the camera can difficult the identification of some species and could produce lower richness values because of missing some characteristic species. Furthermore, there is a "canopy effect" where frondose algae camouflage lower strata of bare rock, encrusting algae and turf forming algae (Álvaro *et al*., 2008; Parravacini *et al*., 2009). These limitations affect the final assessment of the CFR index, as lower richness values produce lower CFR values.

The same limitations can be applied to opportunistic species, because they are usually low-bearing algae that might be very difficult to identify. The exception would be the green opportunistic algae, such as *Ulva spp*. that can be easy to identify to genus level. This would be enough for the application of the CFR index. However, the identification of red or brown opportunistics, such as certain ceramiaceae or ectocarpaceae, can be very difficult to determine from photographs, videos or mosaics. In the present study, the identification of the red opportunistic filamentous *T. intricata* and *F. rufolanosa* was carried out in the laboratory after a collection of a quantitative sample. The great abundance of this species covering most of the seafloor motivated its sampling and identification in the laboratory. However, small abundances of this kind of species could have been disregarded in Castro and Callejos de Bamboa, where no opportunistic species were identified.

Despite the inconveniences mentioned above, the results obtained by the application of the CFR index using mosaics were highly correlated with the results obtained by the direct applications *in situ*. In addition, the assignation of quality classes obtained an "excellent" agreement level in the weighted kappa analysis, thus demonstrating the suitability of the proposed methodology. It must be mentioned that the mosaicing method has demonstrated the same capacity than the direct application in order to detect anthropogenic disturbances, covering the whole range of quality categories from "high" to "bad". The disagreements observed in the assignations of quality classes occurred in 7 out of 19 assessments (Figure 5), but only 2 of these misclassifications produced an important error for management purposes (e.g. the application of the WFD), as their quality assignments corresponded to a "moderate" category in the visual estimation and to a "good" category in the case of the mosaic application, what constitutes a big difference in terms of accordance with the WFD requirements accomplishment, although the difference in terms of CFR values was small. The other 5 misclassifications corresponded to stations located in Ontón which were classified as "poor" by the visual assessment and as "bad" in the mosaic assessment, what has the same meaning to the effects of the WFD, as none of them would achieve an acceptable level.

5. Conclusions

The construction of video mosaics constitute a useful tool for the study of subtidal environments as they provide a wide vision of the seafloor, with reduced efforts and costs for the surveys, specially if they are carried out with ROVs. However, any seafloor video-mosaic application is limited in terms of image resolution and "canopy effect", both of which complicates the identification of some species. The CFR index has been successfully applied to video mosaics that were obtained at three subtidal zones and the results demonstrate the suitability of this technique for the assessment of subtidal communities in extensive monitoring studies. A minimum sampling area of 2.5 m^2 is suggested for the application of the CFR index in subtidal areas using the proposed methodology.

Acknowledgements

This research was partially funded by the Spanish Ministry of Science and Innovation (VIGES2 project, CTM2008-04649) and by the University of Cantabria and the Education Department of the Government of Cantabria trough a postdoctoral research contract to Xabier Guinda. It was also possible thanks to a "José Castillejo" postdoctoral mobility fellowship provided to X. Guinda by the Spanish Ministry of Education. We wish to thank Gorka Bidegain and Beatriz Echavarri for specific support in the fieldwork.

References

Álvaro, N.V., Wallenstein, F.M., Neto, A.I., Nogueira, E.M, Ferreira, J., Santos, C.I., Amaral, A.F., 2008. The use of digital photography for the definition of coastal biotopes in Azores. Hydrobiologia, 596:143-152.

Ballesteros, E., Torras, X., Pinedo, S., García M., Mangialajo, L., De Torres, M., 2007. A new methodology based on littoral community cartography for the implementation of the European Water Framework Directive. Marine Pollution Bulletin, 55: 172-180.

Bermejo, R., Vergara, J. J., Hernández I., 2012. Application and reassessment of the reduced species list index for macroalgae to assess the ecological status under the Water Framework Directive in the Atlantic coast of Southern Spain. Ecological Indicators, 12: 46-57

Bussotti, S., Terlizzi, A., Fraschetti, S., Belmonte, G., Boero, F., 2006. Spatial and temporal variability of sessile benthos in shallow Mediterranean marine caves. Marine Ecology Progress Series, 325: 109-119.

Carpentier, A., Artero, C., Ouisse, V., Perrin, B., Ysnel, F., 2011. Monitoring the subtidal macroalgae percentage cover in an assessment of the human impact on diving sites in Brittany (France): direct estimation versus photography. Cah. Biol. Mar, 52: 247-252.

Castanedo, S., Medina, R., Losada, I.J., Vidal, C., Méndez, F.J., Osorio, A., Juanes, J.A., Puente, A., 2006. The Prestige oil spill in Cantabria (Bay of Biscay). Part I: Operational forecasting system for quick response, risk assessment and protection of natural resources. Journal of Coastal Research, 22: 1474-1489.

Coles, S.L., Brown, E.K., 2007. Twenty-five years of change in coral coverage on a hurricane impacted reef in Hawai'i: the importance of recruitment. Coral Reefs, 26: 705–717.

Condit, R., Hubbell, S.P., Lafrankie, J.V., Sukumar, R., Manokaran, N., Foster, R.B., Ashton, P.S., 1996. Species-area and species-individual relationships for tropical trees: a comparison of three 50-ha plots. Journal of Ecology, 84: 549–562.

DEFRA, 2004. Understanding of Undesirable Disturbance in the Context of Eutrophication, and Development of UK Assessment Methodology for Coastal and Marine Waters. Napier University for the Department for Environment, Food and Rural Affairs, Edinburgh

Derrien, S., Legal, A., 2010. Quality index of Coastal water subtidal macroalgae of French Channel and Atlantic coast QI SUB-MAC. Contract IFREMER-MNHN, 2010, 37p.

Díez, I., Bustamante, M., Santolaria, A., Tajadura, J., Muguerza, N., Borja, A., Muxika, I., Saiz-Salinas, J. I., Gorostiaga, J. M., 2012a. Development of a tool for assessing the ecological quality status of intertidal coastal rocky assemblages, within Atlantic Iberian coasts. Ecological Indicators, 12: 58-71.

Díez, I., Muguerza, N., Santolaria, A., Ganzedo, U., Gorostiaga, J.M., 2012b. Seaweed assemblage changes in the eastern Cantabrian Sea and their potential relationship to climate change. Estuarine, Coastal and Shelf Science, 99: 108-120.

Díez, I., Santolaria, A., Gorostiaga, J.M., 2010. Different levels of macroalgal sampling resolution for pollution assessment. Marine Pollution Bulletin, 60: 1779–1789.

Ducrotoy, J.P., Simpson, S.D., 2001. Developments in the application of photography to ecological monitoring, with reference to algal beds. Aquatic Conserv: Mar. Freshw. Ecosyst, 11: 123–135.

Ellis, D., 1985. Taxonomic sufficiency in pollution assessment. Marine Pollution Bulletin, 16 (12): 459.

Evans, F.C., Clark, P.J., Brand, R.H., 1955. Estimation of the number of species present on a given area. Ecology, 36: 342–343.

Fernández, C., 2011. The retreat of large brown seaweeds on the north coast of Spain: the case of *Saccorhiza polyschides*. European Journal of Phycology, 46 (4): 352-360.

Ferraro, S.P., Cole, F.A., 1990. Taxonomic level and sample size sufficient for assessing pollution impacts on the Southern California Bight macrobenthos. Marine Ecology Progress Series, 67: 251–262.

García-Castrillo, G., Rodríguez, J., Puente, A., Preciado, I., Juanes, J.A., 2000. Cartografiado bentónico sublitoral de la isla de Mouro (Cantabria). Ozeanografika, 3: 69–83.

Garrabou, J., Ballesteros, E*,* Zabala*,* A., 2002. Structure and Dynamics of North-western Mediterranean Rocky Benthic Communities along a Depth Gradient. *Estuarine*, Coastal and Shelf Science, 55: 493–508.

Gleason, A., Lirman, D., Williams, D., Gracias, N., Gintert, B., Madjidi, H., Reid, R., Boynton, G., Negahdaripour, S., Miller, M., Kramer, P., 2007. Documenting hurricane impacts on coral reefs using two-dimensional video-mosaic technology. Marine Ecology, 28: 254- 258.

Gotelli, N. J., Colwell, R. K., 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecological Letters, 4: 379–391.

Gracias, N. and Santos-Victor, J., 1998. Automatic mosaic creation of the ocean floor. OCEANS'98 Conference Proceedings, vol. 1, pp. 257-262.

Guidetti, P., Bianchi, C.N., Chiantore, M., Schiaparelli, S., Morri, C., Cattaneo-Vietti, R., 2004. Living on the rocks: substrate mineralogy and the structure of subtidal rocky substrate communities in the Mediterranean Sea. Marine Ecology Progress Series, 274: 57–68.

Guinan, J., Grehan, A.J., Dolan, M.F.J., Brown, C., 2009. Quantifying relationships between video observations of cold-water coral cover and seafloor features in Rockall Trough, west of Ireland. Marine Ecology Progress Series 375, 125–138.

Guinda, X., Juanes, J.A., Puente, A., Echavarri-Erasun, B., 2012. Spatial distribution pattern analysis of subtidal macroalgae assemblages by a non-destructive rapid assessment method. Journal of Sea Research, 67: 34–43.

Holmes, K.W., Van Niel, K.P., Radford, B., Kendrick, G.A., Grove, S.L., 2008. Modelling distribution of marine benthos from hydroacoustics and underwater video. Continental Shelf Research, 28: 1800-1810.

ICES, 2001. Report of the Working Group on Marine Habitat Mapping. International Council for the Exploration of the Sea-Marine Habitat Committee, Galway, Ireland.

Ierodiaconou, D., Monk, J., Rattray, A., Laurenson, L., Versace, V.L., 2011. Comparison of automated classification techniques for predicting benthic biological communities using hydroacoustics and video observations. Continental Shelf Research, 31: S28-S38.

Jerosch, K., M. Schluter and R. Pesch. 2006. Spatial analysis of marine categorical information using indicator kriging applied to georeferenced video mosaics of the deep-sea Hakon Mosby Mud Volcano. Ecological Informatics, 1(4): 391-406.

JRC, 2011. WFD Intercalibration Phase 2: Milestone 6 report. Coastal Water/ NEA-GIG/ Macroalgae and Angiosperms. Joint Research Centre, European Commission.

Juanes, J.A., Guinda, X., Puente, A., Revilla, J.A., 2008. Macroalgae, a suitable indicator of the ecological status of coastal rocky communities in the NE Atlantic. Ecological Indicators, 8: 351–359.

Kaiser, M.J., 2003. Detecting the effects of fishing on seabed community diversity: importance of scale and sample size. Conservation Biology, 17: 512–520.

Keating, K.A., Quinn, J.F., Ivie, M.A., Ivie, L., 1998. Estimating the effectiveness of further sampling in species inventories. Ecological Applications, 8: 1239 - 1249.

Kollmann, H., Stachowitsch, M., 2001. Long- Term Changes in the Benthos of the Northern Adriatic Sea: A Phototransect Approach. Marine Ecology, 22 (1-2): 135-154.

Lirman, D., Gracias, N., Gintert, B., Gleason, A.C.R., Deangelo, G., Dick, M., Martinez, E., Reid, R.P., 2010. Damage and recovery assessment of vessel grounding injuries on coral reef habitats by use of georeferenced landscape video mosaics. Limnology and Oceanography: Methods, 8: 88–97.

Lirman, D., Gracias, N., Gintert, B., Gleason, A., Reid, R., Negahdaripour, S., Kramer, P., 2007. Development and application of a video-mosaic survey technology to document the status of coral reef communities. Environmental Monitoring and Assessment, 125: 59- 73.

Lorance, P. and V.M. Trenkel. 2006. Variability in natural behaviour, and observed reactions to an ROV, by mid-slope fish species. Journal of Experimental Marine Biology and Ecology, 332(1): 106-119.

Ludvigsen, M., Sortland B., Johnsen G., Hanumant, S., 2007. Applications of Geo-Referenced Underwater Photo Mosaics in Marine Biology and Archaeology. Oceanography, 20 (4): 140-149.

Marks, R.L., Rock, S.M., Lee, M.J., 1995. Real-time video mosaicing of the ocean floor. IEEE Journal of Ocean Engineering, 20 (3): 229-241.

Melo, A.S., Bini, L.M., Thomaz, S.M., 2007. Assessment of methods to estimate aquatic macrophyte species richness in extrapolated sample sizes. Aquatic Botany, 86: 377–384.

Melo, A.S., Pereira, R.A.S., Santos, A.J., Shepherd, G.J., Machado, G., Medeiros, H.F., Sawaya, R.J., 2003. Comparing species richness among assemblages using sample units: why not use extrapolation methods to standardize different sample sizes? Oikos, 101: 398–410.

Monserud, R., Leemans, R., 1992. Comparing global vegetation maps with the Kappa statistic. Ecological Modelling, 62: 275–293.

Neto, J.M., Gaspar R., Pereira, L., Marques J.C., 2012. Marine Macroalgae Assessment Tool (MarMAT) for intertidal rocky shores. Quality assessment under the scope of the European Water Framework Directive. Ecological Indicators, 19: 39-47.

Norcross, B.L. and F.J. Mueter. 1999. The use of an ROV in the study of juvenile flatfish. Fisheries Research, 39(3): 241-251.

Norris, J. G., Wyllie-Echeverria, S., Mumford, T., Bailey, A., Turner, T.,1997. Estimating basal area coverage of subtidal seagrass beds using underwater videography. Aquatic Botany, 58: 269-287.

Orfanidis, S., Panayotidis, P., Stamatis, N., 2003. An insight to the ecological evaluation index (EEI). Ecological Indicators, 3: 27-33.

Parravicini, V., Micheli, F., Montefalcone, M, Villa, E., Morri, C, Bianchi, C.N., 2010. Rapid assessment of epibenthic communities: A comparison between two visual sampling techniques. Journal of Experimental Marine Biology and Ecology, 395: 21–29.

Parravicini, V., Morri, C, Ciribilli, G., Montefalcone, M., Albertelli, G.,Bianchi, C.N., 2009. Size matters more than method: Visual quadrats vs photography in measuring human impact on Mediterranean rocky reef communities. Estuarine, Coastal and Shelf Science, 81: 359–367.

Pinedo, S., García, M., Satta, M.P., de Torres, M., Ballesteros, E., 2007. Rocky-shore communities as indicators of water quality: A case study in the Northwestern Mediterranean. Marine Pollution Bulletin, 55: 126–135.

Puente, A., Juanes, J.A., 2008. Testing taxonomic resolution, data transformation and selection of species for monitoring macroalgae communities. Estuarine, Coastal and Shelf Science, 78: 327–340.

Rooper, C.N., Zimmermann, M., 2007. A bottom-up methodology for integrating underwater video and acoustic mapping for seafloor substrate classification. Continental Shelf Research, 27: 947–957.

Rzhanov, Y., Linnett, L.M., Forbes, R., 2000. Underwater video mosaicing for seabed mapping. In Proceedings of ICIP 2000.

Rzhanov, Y., Mayer, L., Beaulieu, S., Soule, S.A., Shank, T. 2007. The generation of georeferenced video mosaics in support of submersible and ROV operations. CenSeam Standardisation Working Group and Data Analysis Working Group meeting, Dartington Hall, Plymouth, UK, 2007.

Smale, D.A., 2008.Continuous benthic community change along a depth gradient in Antarctic shallows: evidence of patchiness but not zonation. Polar Biology, 31: 189–198.

Somerfield, P.J., Clarke, K.R.., 1995. Taxonomic levels in marine community studies revisited. Marine Ecology Progress Series, 127: 113–119.

Søreide, F., Jasinski, M.E., 2005. Ormen Lange: Investigation and excavation of a shipwreck in 170-m depth. Pp. 2,334–2,338 in *Proceedings of OCEANS 2005, Washington DC, September 18–23, 2006, Volume III.* Marine Technology Society and Institute of Electrical and Electronics Engineers.

Valencia, V., Borja, A., Franco, J., Fontán, A., 2004. Hydrography of the southeastern Bay of Biscay, in: Borja, A., Collins, M (Ed.), Oceanography and marine environment of the Basque Country. Elsevier, pp. 159-194.

Van Rein, H., Schoeman, D.S., Brown, C.J., Quinn, R., Breen, J., 2011. Development of benthic monitoring methods using photoquadrats and scuba on heterogeneous hard-substrata: a boulder-slope community case study. Aquatic Conserv: Mar. Freshw. Ecosyst, 21: 676–689

Walther, B.A., Cotgreave, P., Price, R.D., Gregory, R.D., Clayton, D.H., 1995. Sampling effort and parasite species richness. Parasitology Today, 11: 306–310.

Warwick, R.M., 1988a. Analysis of community attributes of the macrobenthos of Frierfjord/ Langesundfjord at taxonomic levels higher than species. Marine Ecology Progress Series, 46: 167-170.

Warwick, R.M., 1988b. The level of taxonomic discrimination required to detect pollution effects on marine benthic community. Marine Pollution Bulletin, 19: 259-268.

Wells, E., Wilkinson, M., Wood, P., Scanlan, C., 2007. The use of macroalgal species richness and composition on intertidal rocky shores in the assessment of ecological quality under the European Water Framework Directive. Marine Pollution Bulletin, 55: 151-161.