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Journal of Experimental Marine Biology and Ecology 418–419 (2012) 75–82

Contents lists available at SciVerse ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

A rapid photographic method detects depth gradient in coralligenous assemblages

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ARTICLE INFO

Article history:
Received 25 November 2011
Received in revised form 8 March 2012
Accepted 9 March 2012
Available online xxxx

Keywords:
Benthic organisms
CPCe
Hard bottom assemblage
Photographic quadrat
Rocky habitat
Sampling technique

ABSTRACT

Coralligenous assemblages represent the unique calcareous formations of biogenic origin in the Mediterranean Sea. Despite their importance in terms of biodiversity and biomass production, community analyses remain scarce. Actual sampling of these assemblages is complicated to carry out because their depth distribution (down to −120 m) necessitates complex diving logistics. We highlight a rapid, cost-effective, objective and accurate method for the sampling of coralligenous assemblages and tested its efficiency in delineating a depth gradient. We compared seven photographic methods for estimating the percentage cover of sessile organisms: visual estimates (VS) with the aid of a 25, 64 or 100 square-grid and random-point-quadrats (RQ) with 25, 64 or 100 random points or 64 stratified random points. Comparisons were made using two simulated quadrats for which percent cover values were known. RQ with 64 random points was the method that accumulated the highest number of advantages. Using this method, two field sites were sampled by divers at three depths (−50, −60 and −70 m) with increasing replication (10, 20, 30 and 40 photographic quadrats). The communities deduced from the 30 and 40 photos were similar. Community analyses showed an effect of depth nested in site on the assemblages observed. With increasing depth, encrusting algae get replaced by Porifera. Dissimilarity between −50 m and −60 m/−70 m was mainly due to *Crambe tailliezi* abundance. This methodology will be a useful tool for managers and administrators; it guarantees fast abundance estimation, non-destructive repeated sampling, the possibility of comparison among researchers and the permanent record of deep-sea communities.

1. Introduction

After *Posidonia oceanic* meadows, "coralligenous assemblages" are considered as the second biodiversity hot spot in the Mediterranean Sea (Boudouresque, 2004). Coralligenous assemblages colonize the deep littoral systems of the Mediterranean Sea from 12–50 m to 40–120 m depth depending on water transparency (Ballesteros, 2006). Coralligenous concretions are primarily produced by the accumulation of encrusting algae growing at low light levels and secondarily by bio-constructor animals such as polychaetes, bryozoans and gorgonians; they represent the unique calcareous formations of biogenic origin in the Mediterranean Sea (Ballesteros, 2006). The resulting complex structure allows the development of a patchwork of communities dominated by living algae, suspension feeders, borers or soft-bottom fauna (in the sediment within cavities). Two main morphologies can be distinguished (Ballesteros, 2006) for coralligenous frameworks: banks (built over more or less horizontal substrata) and rims (in the outer part of marine caves and on vertical cliffs). In terms of richness, biomass and production, coralligenous assemblage value is high and comparable to tropical reef assemblages (Bianchi, 2001). The engineering species composing these assemblages are fragile, present long life expectancy and low dynamics leading to a particular susceptibility of coralligenous assemblages (in their specific composition and structure) to anthropogenic disturbances (waste water, invasive species, fishing activities and divers) (Ballesteros, 2006). Identified under Community legislation (Habitats Directive 92/43/CEE; habitat code 1170–14: Reefs, coralligenous assemblage) as being a special habitat with biodiversity interest, coralligenous assemblages shall be monitored as a descriptor in the framework of Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC). MSFD aims to achieve good environmental status of the EU's marine waters by 2020 and to protect the resource base upon which marine-related economic and social activities depend. The final goal is to protect more effectively the marine environment across Europe but an important point is that each Member State "shall ensure that measures are cost-effective and technically feasible".

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doi:10.1016/j.jembe.2012.03.006

Action plans for the conservation of the coralligenous and other calcareous bio-concretions highlighted a lack of standardized procedures for characterizing these habitats (UNEP-MAP-RAC/SPA, 2008, 2011). Moreover, Kipson et al. recently claimed that "methods are urgently needed to assess prevailing patterns, evaluate impacts to which they [coralligenous outcrops] are subjected and provide baseline data to explore future trajectories of these high diversity assemblages" (Kipson et al., 2011). For these reasons and because they cumulate study constraints such as various systems, slow dynamics, and more importantly presence at generally high depths limiting work time underwater, coralligenous assemblages are relatively poorly understood at the community level (Kipson et al., 2011). Saving time underwater is one of the advantages of photographic methods that are more and more frequently used (Balata et al., 2005; Baldacconi and Corriero, 2009; Deter et al., 2012; Ferdeghini et al., 2000; Kipson et al., 2011; Virgilio et al., 2006). They allowed counting the number of species and/or estimating species abundance using percent cover. Percent cover is used to quantify organisms that cover the substrate and have modular body organization, including most macroalgae, sponges and colonial animals such as corals, bryozoans and ascidians (Benedetti-Cecchi et al., 1996; Boudouresque, 1971). Feasibility and cost-efficiency but also rapidity, objectivity, repeatability, accuracy and sensitivity are all important criteria to be considered in the choice and/or development of a monitoring method.

Percent cover estimated from digitized images produced similar results (with equivalent quadrat size) (Parravicini et al., 2009) and were more repeatable and objective than direct estimates in the field (Meese and Tomich, 1992). This is especially true for the random-point method (described below) (Alquezar and Boyd, 2007). Despite the risk of misidentification, the main advantage recognized for digital methods is its rapidity (Macedo et al., 2006). However the necessity to spend time in the laboratory for the processing of pictures may discourage its employment for monitoring. Thus, the challenge lies in finding a good balance between time constraints (both underwater and in the laboratory), cost and efficiency by identifying a reliable technique for picture processing and assessing the replication effort needed.

Two methods are generally used for abundance estimation from photographic quadrats: visual methods (VS) and random-point methods (RQ). VS consists of evaluating percent cover with the aid of a grid projected on to the photographic quadrat. With RQ, a specified number of spatially random points are overlaid on the image and the features (species or substrates) lying beneath each point are user-identified. Percent cover is then calculated from the different number of points identified per species. With manual projections (grid or points on transparencies) both methods took comparable amounts of time and were similarly repeatable for abundant species (>10%) (Dethier et al., 1993). Thanks to CPCe (Coral Point Count with Excel extensions), a user-friendly software (Kohler and Gill, 2006), RQ is now easier to perform. This software reduces the time spent (image preparation and analysis) for RQ by grouping in a unique interface picture enhancement, point distribution and identification. Percent cover is then calculated and the results sent to the Excel spreadsheet automatically. First developed for coral reef studies, CPCe is easily adaptable to other assemblages by recoding the species to be identified (Kohler and Gill, 2006); it was consequently also applied to artificial habitats, rocky intertidal assemblage, and coralligenous assemblages sampling (Macedo et al., 2006; Márquez I Canals, 2006; Pineda, 2007; Zintzen et al., 2008).

The present study aimed at testing and comparing the rapidity (time required), objectivity (variability among observers), repeatability (within-observer variance), accuracy (divergence of the results from the real values) and sensitivity (proportions of missed species occurrences, especially the rarest ones) between VS and RQ (with different number of points and type of projection) using simulated digitized photos. We tested the hypotheses that RQ was faster, more objective and more repeatable than VS for equivalent accuracy and sensitivity. Secondly, we evaluated the performance of the best method combination (method × number of points, selected from preceding tests) in the field to detect a depth gradient, and the number of pictures required (sampling effort needed).

2. Materials and methods

2.1. Methods tested with simulated quadrats

Two 0.25 m^2 quadrats (50 × 50 cm) were created using The GIMP (The GIMP Team, 2010. GNU Image Manipulation Program, www.gimp.org) 2.6 software to draw the distribution of nine and eleven "species" (one color per species, Table 1). The first simulated quadrat (quadrat A) mimicked highly interlocked distributions (Fig. 1A) while the second one (quadrat B) represented more patchy distributions (Fig. 1B). Three rearranged versions of each quadrat were created showing the same number of species and surface coverage but at different dispositions.

The two methods were tested on each simulated quadrat for the percent cover estimation (species cover in %): the visual method (VS) and the random-point-quadrat method (RQ). For VS, each photographic quadrat was divided into x small squares with the aid of a grid superimposed on the quadrat frame using The GIMP software. Species were identified and the percent cover estimated with the help of the grid: each small square totally filled by a species counted as $100/x$% cover, a square 3/4 filled count for $75/x$%, half filled for $50/x$% cover, etc. This mode eliminates the need for decision rules such as any square > half filled is counted as filled (Dethier et al., 1993). For RQ, CPCe 2.6 (Kohler and Gill, 2006) was used to project a number of points within the photographic quadrat frame. The distribution of these points was either totally random (no preferential spatial distribution of y points) or stratified random (projection of one point per cell within a grid of z cells dividing the quadrat frame). Species underlying the points were identified and percent covers automatically calculated.

Three observers analyzed the first version of quadrat A with VS (aid of $x = 25$, 64 or 100 squares) and with RQ (aid of $y = 25$, 64 or 100 random points or $z = 64$ stratified random points). To avoid a possible bias due to user habit, all three observers were marine naturalists but non-trained to one or the other method. The number of 64 points was chosen after tests on three representative field quadrats

Table 1
Abundances (percent covers) and species represented on quadrats A and B.

Species	Percent cover
Quadrat A	
a	3.17
b	4.88
c	4.39
d	7.49
e	8.52
f	8.57
g	11.18
h	17.75
i	34.05
Quadrat B	
j	0.50
k	1.00
b	1.50
l	2.00
e	3.25
f	4.75
m	8.00
a	10.00
h	17.00
n	21.00
c	31.00

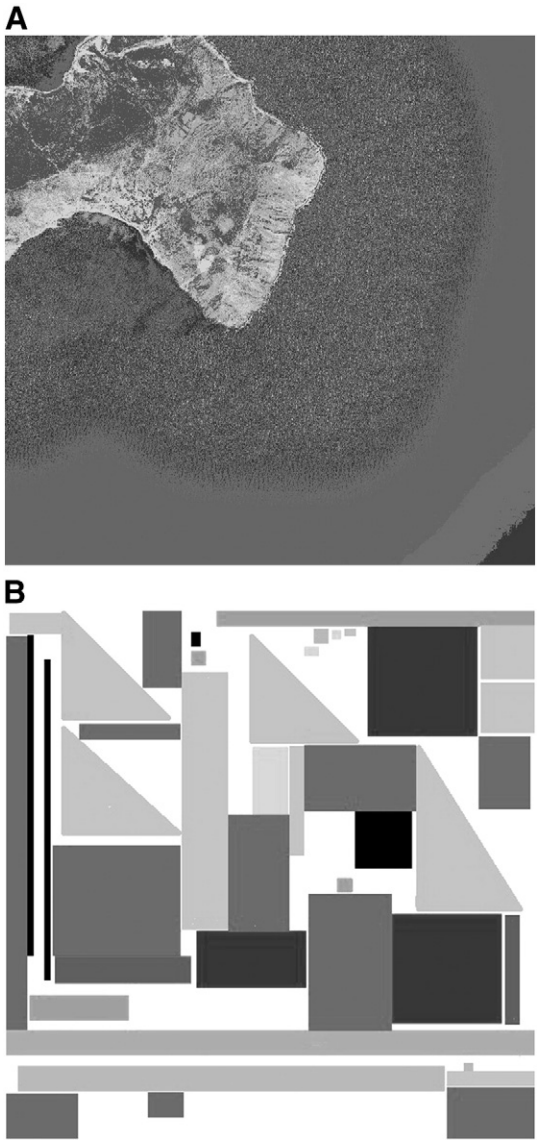

Fig. 1. First version of the simulated quadrats transformed in black and white for the publication. Quadrat A (A) mimicked a highly interlocked distributions of nine "species" when quadrat B (B) represented eleven "species" with patchy distributions (one color per species).

and varying numbers of points (9, 16, 25, 36, 49, 64, 81, 100, 144, 169, 196, 225 and 256 points per quadrat). Sixty-four points were needed to identify all the recognizable features of the pictures as more points did not add any more information. The numbers 25 and 100 were chosen to frame 64. Each combination (number of points × method) was performed three times per observer (three observers in total) once a week to avoid biases due to the observer remembering previous estimates. For the same reason, observers were not informed of the total number of simulated species. The time required for each quadrat sampling per method was noted. Rapidity (time required) and accuracy (divergence of the results from the real values = absolute value of the difference between observed and real values) were compared between groups of methods (two: VS and RQ) and observers (three) using analyses of variance (ANOVA) performed for repeated measures (three times). When a significant difference was found between groups of methods, post hoc tests (Kolmogorov–Smirnov tests or *t*-tests) were performed to highlight differences between combinations (number of points × method).

All dependent variables were normalized with adequate transformations. Differences in sensitivity (proportions of missed species occurrences) between the methods were tested with 2 × 2 contingency tables. Objectivity (variance between observers) was tested with non-parametric tests (*t*-tests).

The best combinations (method × number of points–squares) were tested by observer 1 with all versions of quadrats A and B added to the corresponding results obtained by observers 2 and 3 in order to test for the influence of the method and evaluate accuracy and sensitivity more precisely. Repeatability (variance for a same observer) was only tested with results obtained from observer 1 with an ANOVA for repeated measures. All of the statistical analyses were performed with Statistica 6.1 (Statsoft, Inc.).

2.2. Field data sampling

The aim of the field sampling was to test the ability of the method selected from tests with simulated quadrats in detecting a depth gradient and the number of pictures required (sampling effort needed).

Two sites localized in the Mediterranean Sea next to Fréjus (Var, France. Fig. 2) were sampled in June 2010 at three different depths (−50 m, −60 m and −70 m): Banc des vieilles (43°24′44″; 6°53′593″) and Chrétienne (43°26′371″; 6°55′443″). Both of these sites present similar coralligenous assemblages patterns (rim morphology according to Ballesteros, 2006) developed on vertical cliffs from −71 to −46 m at Banc des vieilles and from −70 to −50 m at Chrétienne. These sites were chosen because a depth gradient was clearly visible in assemblages according to the divers. The same diver took photos along a 20 meter-transect at each site. For practical and safety reasons, dives were performed using rebreathers INSPIRATION VISION (Ambiant Pressure Diving Ltd., United Kingdom). Gas recycling reduces the volume of breathing gas used, making longer dives possible with a lighter and more compact system than an open-circuit breathing set for the same duration (Bahuet et al., 2007).

Around 45 photographic quadrats per site were taken along the 40 m-transects at each depth (360 photos in total). One photo per 50 × 50 cm quadrat was taken using a digital camera (D2Xs Nikon at 12.4 megapixels with a 12–24 mm zoom-lens Nikon equipped with a housing, a dome and flashes SEACAM®). The camera was perpendicularly fixed 50 cm over the quadrat frame, thus minimizing possible parallax errors (Fig. 3). A compass and a depth meter were attached to the quadrat in order to keep homogenous the orientation (North at 12 h) and depth for the photos.

Forty photographic photos per depth within each site were randomly selected using the program Randomize (M. Rolland — Andromede

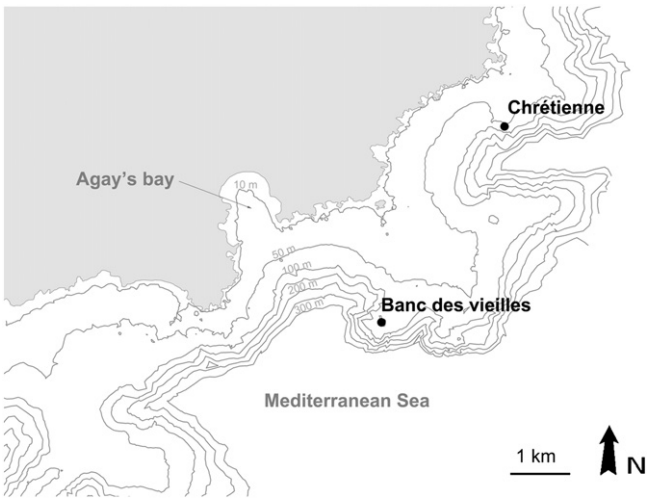

Fig. 2. Map presenting the field sites localized in Mediterranean Sea next to Fréjus (Var, France): Banc des vieilles (43°24′44″; 6°53′593″) and Chrétienne (43°26′371″; 6°55′443″). They were sampled in June 2010 at three different depths (−50 m, −60 m and −70 m).

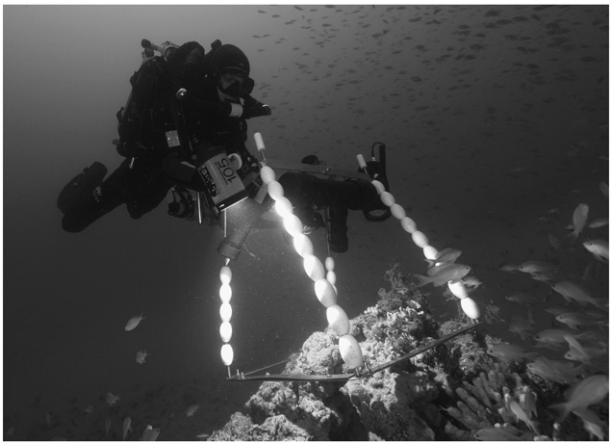

Fig. 3. Photographic quadrat system used in the field. A digital camera was perpendicularly fixed 50 cm over the quadrat frame.

Oceanology) and enhanced for quality (contrast, luminosity, and sharpness) with The GIMP 2.6. The best method selected from simulated images was performed on the 40 photographic quadrats but also on 30, 20 and 10 of these images randomly chosen by the program Randomize.

Taxa (sessile organisms) were identified to the level of species or genus. Taxonomic nomenclature follows Appeltans et al. (2011) and Guiry and Guiry (2011). Where identification at the most detailed level of taxonomical resolution was not possible, animals were grouped in phyla. Hydrozoa and encrusting Bryozoa were not identified and classified as "Hydrozoa" and "Encrusting Bryozoa". Unidentifiable organisms were classified as "unknown" and were not considered in community analyses. Similarly, mobile organisms (fish, urchins) were not considered in community analyses. The matrix of species percent cover obtained on the base of the different number of photos was fourth-root transformed (because the data set was strongly dominated by certain variables) and analyzed with Primer 6.1.11 software (Primer-E), according to Clarke and Warwick (2001), Clarke and Gorley (2006) and Clarke et al. (2008). Similarity between samples was estimated using Bray–Curtis indices. A SIMPER breakdown was performed to determine the species that mostly contribute to the average similarity/dissimilarity within and between sites and depths. The existence of a depth gradient in species assemblages was tested using an ANOSIM; ANOSIM (two-way nested analysis of similarities) was performed on a similarity matrix and tested for a significant difference between sites and between depths within sites. The procedures SIMPROF and CLUSTER (hierarchical cluster analyses) were performed in order to test and visualize differences between the assemblages obtained from the different numbers of photos (10, 20, 30 or 40) and deduce the minimal number of pictures that did not change assemblage results (sampling effort needed). *P*-values were obtained with permutation tests (10,000 permutations).

3. Results

3.1. Testing the methods with simulated quadrats

3.1.1. Rapidity

All number of points–squares confounded (25, 64 or 100), ANOVA with repeated measures ($N=21$ observations repeated three times) showed a significant effect of the method used ($F=12.677$, $P=0.001$) while no effect of the observer ($F=2.023$, $P=0.099$) or of the interaction observer × method ($F=1.244$, $P=0.317$) was significant. VS was the slowest type of method (Table 2). Taking the number of points into account, RQ with 25 points was the fastest (mean = 65 ± 22 s), followed by RQ with 64 points (no difference between totally random (190 ± 18 s) or stratified random (183 ± 52 s), Kolmogorov–Smirnov test, $P=0.100$) (Fig. 4A). RQ with 64 points was faster than VS with 25 squares (Kolmogorov–Smirnov test, all $P<0.010$).

Table 2
Mean results (standard deviation = SD in parenthesis) obtained for each combination tested (number of points–squares × method). RQ = random-point quadrat with 25, 64 or 100 S = stratified or R = random points; VS = visual method with the aid of 25, 64 or 100 squares. All data were pooled (quadrat A with three observers). Rapidity referred to the amount of time required in seconds, sensitivity to the number of missed species on the number of occurrence, accuracy to the divergence from real values and objectivity to the variance of estimated percent covers between the observers.

	Rapidity	Sensitivity	Accuracy	Objectivity
RQS64	182.78 (52.28)	2/81	7.73 (6.54)	4.38 (5.43)
RQR25	65.67 (22.33)	12/81	3.10 (2.46)	3.453 (3.22)
RQR64	190.33 (18.15)	3/81	3.45 (3.10)	1.78 (1.31)
RQR100	412.33 (194.65)	1/81	3.25 (3.48)	1.439 (1.31)
VS25	485.00 (116.00)	5/81	5.11 (5.64)	8.91 (9.98)
VS64	1147.67 (399.54)	5/81	2.94 (2.84)	12.26 (20.66)
VS100	1200.67 (750.69)	3/81	1.78 (0.83)	19.92 (25.58)

3.1.2. Sensitivity

No difference in sensitivity was found between the methods (18/324 for RQ with 2/81 for RQ with stratified random points and 16/243 with totally random points and 13/243 with VS, 2 × 2 contingency table, all $P>0.566$). When species were missed, real percent cover was <5%. RQ with 25 random points was the combination point–method that missed the highest proportion of species (12/81)

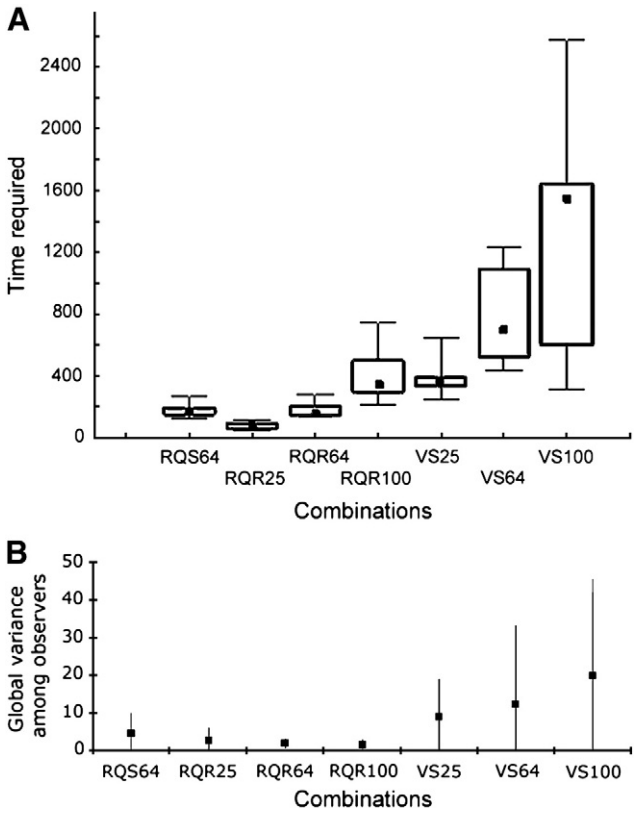

Fig. 4. Comparison of the results obtained from the first version of quadrat A analyzed three times per each observer for each combination (number of points–squares × method). A) Time required (in seconds) for the analysis. Black squares represented the median of the data, the box 25 and 75% of the data distribution and horizontal traits maximal and minimal values obtained on three observers. B) Global variance (for all species) among observers in percent cover estimation. Vertical traits represent standard deviation. RQ = random-point quadrat with 25, 64 or 100 S (= stratified) or R (= random) points; VS = visual method with the aid of 25, 64 or 100 squares. $N=189$ observations.

Table 3
List of taxa found at Banc des vieilles (B) and Chrétienne (C) at three different depths (−50, −60 and −70 m).

Taxa (alphabetical order)	Observed at …
Annelida Cl. Polychaeta	
Filograna implexa Berkeley, 1835 or *Salmacina* sp.	B60, C60
Other worms	All sites and depths
Bryozoa cl. Gymnolaemata	
Adeonella calveti Canu and Bassler, 1930	B70 and C50
Encrusting Bryozoa	C70
Myriapora truncata Pallas, 1766	B50, B60, B70, C50, C60
Non identified Bryozoa	All sites and depths
Pentapora fascialis Pallas, 1766	C50
Reteporella grimaldii Jullien, 1903	B60, B70, C50, C60, C70
Smittina cervicornis Pallas, 1766	B50, B60
Chlorophyta cl. Bryopsidophycae	
Caulerpa racemosa f. *compressa* Weber-van Bosse, 1898	B50, C50, C70
Codium bursa (Olivi) C. Agardh, 1817	C50
Codium coralloides (Kützing) P.C. Silva, 1960	B50, C50, C60
Flabellia petiolata (Turra) Nizamuddin, 1987	B50, C50
Halimeda tuna (J. Ellis & Solander) J.V. Lamouroux, 1816	C50
Non identified macroalgae	All sites and depths
Chordata cl. Ascidiacea	
Ciona intestinalis Linnaeus, 1767	C60
Halocynthia papillosa Linnaeus, 1767	C50
Microcosmus sabatieri Roule, 1885	C50
Non identified Ascidiacea	All sites and depths
Phallusia fumigata Grube, 1864	C60
Polycitor sp.	C50 and C60
Cnidaria Cl. Anthozoa	
Alcyonium corraloides Pallas, 1766	B50, B60, B70, C50
Alicia mirabilis Johnson, 1861	C60
Astroides calycularis Pallas, 1766	B70
Corallium rubrum Linnaeus, 1758	B60, B70, C60, C70
Eunicella cavolinii Koch, 1887	All sites and depths
Eunicella singularis Esper, 1791	B50, B70, C60
Hoplangia durotrix Gosse, 1860	C60, C70
Leptogorgia sarmentosa Esper, 1789	B50, B60, B70, C50, C60
Leptopsammia pruvoti Lacaze-Duthiers, 1897	B60, B70
Non identified Alcyonacea	B60, C50, C60
Non identified Hydrozoanthidae	All sites and depths
Non identified Scleractinia	B60, B70, C70
Non identified Zoanthidea	B60, B70
Non identified Gorgonidae	B50, C70
Paramuricea clavata Risso, 1826	All sites and depths
Parazoanthus axinellae Schmidt, 1862	B50, B60, B70, C50
Foraminifera cl. Polythalamea	
Miniacina miniacea Pallas, 1766	All depths at C
Heterokontophyta cl. Phaeophyceae	
Cystoseira mediterranea Sauvageau, 1912	B50
Porifera cl. Calcarea	
Clathrina coriacea Montagu, 1818	B60, B70, C60
Porifera cl. Demospongiae	
Agelas oroides Schmidt, 1864	All sites and depths
Aplysilla sulfurea Schulze, 1878	B60, B70, C60, C70
Aplysina aerophoba Nardo, 1833	C60
Aplysina cavernicola Vacelet, 1959	B50, B60, B70, C60, C70
Axinella damicornis Esper, 1794	All sites and depths
Axinella polypoides Schmidt, 1862	B50, B60, C50, C70
Axinella vaceleti Pansini, 1984	B60, C60
Axinella verrucosa Esper, 1794	C60
Chondrosia reniformis Nardo, 1847	B50, B70, C70
Cliona schmidti Ridley, 1881	C50, C60
Cliona viridis Schmidt, 1862	All sites and depths
Crambe tailliezi Vacelet & Boury-Esnault, 1982	B60, B70, C60, C70
Crella (*Crella*) *mollior* Topsent, 1925	All depths at C
Diplastrella bistellata Schmidt, 1862	C60
Dysidea avara Schmidt, 1862	B50, B60, B70, C60, C70
Haliclona sp.	B50, B60, B70, C60, C70
Hemimycale columella Bowerbank, 1874	C60
Hexadella racovitzai Topsent, 1896	B50, B70, C50, C60, C70
Ircinia variabilis Schmidt, 1862	B50, B70
Myxilla (*Myxilla*) *incrustans* Johnston, 1842	C70
Non identified Porifera	All sites and depths
Phorbas tenacior Topsent, 1925	All sites and depths
Spirastrella cunctatrix Schmidt, 1868	All sites and depths
Spongia (*Spongia*) *agaricina* Pallas, 1766	C60
Porifera cl. Homoscleromorpha	
Oscarella lobularis Schmidt, 1862	B50, B60, B70, C60, C70
Rhodophyta cl. Florideophyceae	
Amphiroa sp.	C60
Halymenia floresia (*Clemente*) C. Agardh, 1807	C50
Lithophyllum sp. and *Mesophyllum* sp.	All sites and depths
Lithothamnium sp.	C70
Peyssonnelia squamaria (*S. Gmelin*) Decaisne, 1842	B50, B60, C50, C60

contrary to VS with 100 points and RQ with 100 points that detected all species (Table 2).

3.1.3. Accuracy

ANOVA with repeated measures on divergence from real values ($N = 188$ observations repeated three times) did not show any significant effect of the group of methods (all points–squares confounded, $F = 2.219$, $P = 0.041$), the observer ($F = 0.554$, $P = 0.767$) or the interaction observer × method ($F = 0.949$, $P = 0.497$). Divergences in percentage of the real values ranged from 8% (species *i* with RQ and 100 random points) to 100% (species *g* with RQ and 64 stratified random points). A comparison with the real values revealed that percent cover was generally underestimated.

3.1.4. Objectivity

Percent cover showed variance among observers ranging between 0.018 (species *e*, RQ with 25 random points) and 78.801 (species *h*, VS with 100 squares) with the weakest global mean variance (mean of the variance estimated for each species) noted for RQ with 100 (1.439) and 64 (1.780) random points (Table 2, Fig. 4B). The highest variance was mostly observed for VS (regardless of the number of squares used in the grid) and especially for species covering more than 10%. VS was thus the least objective method. The grid of 25 squares produced the weakest variance within the VS method but was nevertheless more variable than RQ with 64 or 100 random points (*t*-test on paired data, $P = 0.036$ and $P = 0.022$ respectively).

3.1.5. Testing the best combinations

Finally, RQ with 64 totally random points was faster than with 100 points while maintaining the same objectivity. VS using 25 squares produced the least variable results with the shortest time compared to other VS combinations. Ranking the methods depending on the results obtained for each measured variable, RQ with 100 random points was the best method followed by RQ with 64 random points. Considering the time required, almost 7 min per quadrat was judged too long for RQ with 100 random points when RQ with 64 random points took around 3 min per quadrat. Following these results, the best trade-off was presented by RQ with totally random 64 points (called RQR64) and VS with the aid of 25 squares (VS25). These were thus tested by observer 1 with all versions of quadrats A and B.

VS25 was globally less accurate than RQR64 ($4.187 > 2.911$, *t*-test: $t = -2.649$, $P = 0.009$). On the contrary, with 12 species missed on 234 occurrences, VS25 was more sensitive than RQR64 (26/234) (2×2 contingency tables, $\chi^2 = 5.61$, $P = 0.178$). Real percent covers of missed species were 3.25% *maximum*. Concerning the repeatability, global variance of the results obtained from observer 1 was 9.787 (SD = 15.943) with RQR64 and 12.920 (SD = 26.657) with VS25 but the difference was not significant (ANOVA on $N = 48$ observations repeated three times, $F = 0.904$, $P = 0.448$).

3.2. Field sampling and depth gradient detection

During the sampling, temperatures were 19 °C at the surface and 14 °C and 16 °C at −70 m respectively at Banc des vieilles and

Chrétienne. Both sites presented a thermocline at −17 m and 10 m visibility. The current was South West at Banc des vieilles and East at Chrétienne. Following the results presented above, the RQR64 method (faster, better objectivity and accuracy) was performed with field data. The mean percentage of surface not covered by sessile organisms at both sites was 35%. A sludge percent was determined from the number of points (with 40 quadrats) projected on sludge; it was 28%, 24% and 42% at Banc des vieilles at −50, −60 and −70 m and 34%, 34% and 30% at Chrétienne at −50, −60 and −70 m. In total, 70 taxa were identified (Table 3). The most abundant species were the Porifera *Aplysina cavernicola* (Vacelet, 1959), the Rhodophyta *Mesophyllum* sp. and *Lithophyllum* sp., the Porifora *Crambe tailliezi* (Vacelet and Boury-Esnault, 1982),the Octocorallia *Paramuricea clavata* (Risso, 1826) and encrusting Bryozoa (Fig. 5). Weak differences were observed between results obtained from the different number of photographic quadrats (Fig. 5); CLUSTER with SIMPROF test showed that results obtained from 30 or 40 quadrats were always similar (Fig. 6) and sometimes not different from 20 quadrats (at Banc des vieilles −60 m and Chrétienne −50 m).

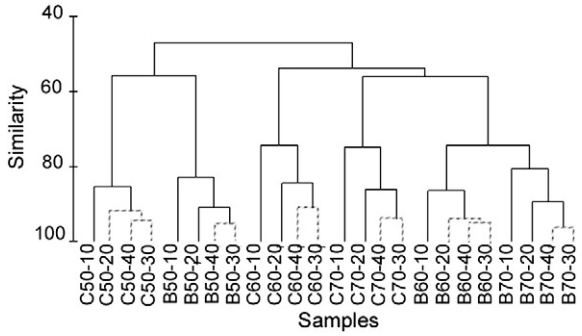

Fig. 6. Graphic representation of the CLUSTER results showing the samples grouped by similarity (Bray–Curtis Index). Continuous line linked dissimilar groups when dotted lines refer to non significant differences. The code used referred to site, depth and number of photos; C50-10 for example indicates a sample from Chrétienne at −50 m on the base of 10 photographic quadrats.

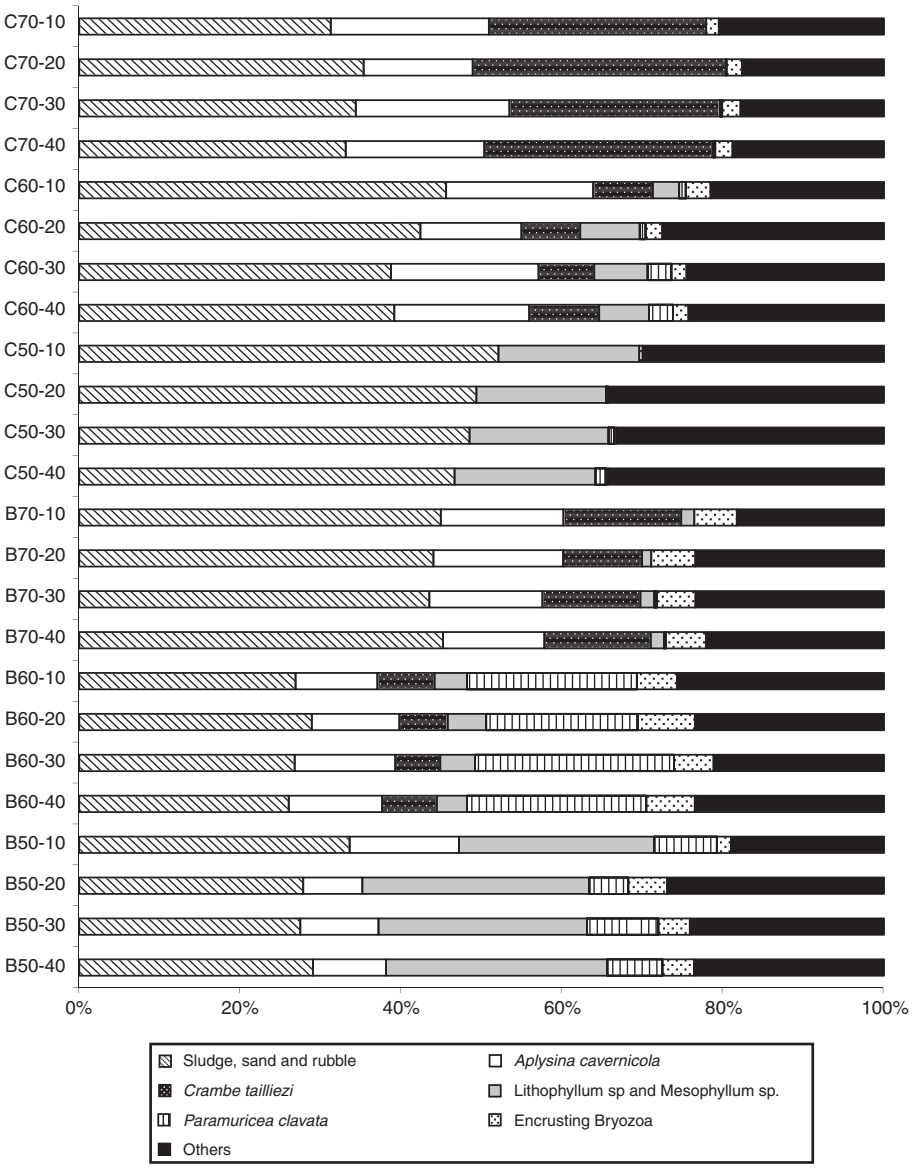

Fig. 5. Distribution of the most abundant species (in percent cover) observed at Banc des vieilles and Chrétienne at −50, −60 and −70 m on the base of 40, 30, 20 and 10 photographic quadrats. The code used referred to site, depth and number of photos; C50-10 for example indicates a sample from Chrétienne at −50 m on the base of 10 photographic quadrats.

Fig. 6 illustrates also how the deepest samples (−60 m and −70 m) were more similar than the shallowest ones. Dissimilarity between −50 m and −60 m/−70 m was mainly due to *C. tailliezi* abundance (SIMPER, contributed to 6.28% of the dissimilarity). Similarity within samples from −50 m was mainly produced by *Lithophyllum* sp. and *Mesophyllum* sp. (SIMPER, contribution of 10.81% to the similarity), from −60 m by *A. cavernicola* (contribution of 8.05%) and from −70 m by *C. tailliezi* (contribution of 9.63%). ANOSIM confirmed these observations: assemblages did not significantly differ between sites (ANOSIM, $P = 0.30$) whereas a highly significant difference was detected between depths within sites (ANOSIM, $P = 0.001$).

4. Discussion

4.1. Comparison between results issued from simulated quadrats: the choice of random-point quadrat method with 64 points

Whatever the method, no difference in sensitivity or accuracy was found. As hypothesized, VS was a far less objective method compared to RQ. In the field, Dethier et al. (1993) reported VS as the most difficult method to perform since "the sampler needs to concentrate on the distributions of various species rather than simply identifying and recording the species under each point". Although they did not test the influence of different grid sizes, they assumed "mental integration" would be easier with a larger relative number of small subdivisions. According to these assumptions, VS using 100 squares tended to be more accurate than VS25. Nevertheless, VS25 was the fastest and most objective VS combination. Whereas most studies only compared random vs. visual sampling, we also tested the use of stratified random points. Expected to avoid potential grouping of points, this combination was strangely the least accurate than the others probably because of a non relevant subdivision in regard to species distributions (non regular). Because of an absence of regularity in distribution patterns, Dethier et al. (1993) preferred to use random rather than systematic points.

Comparing all combinations within RQ, our results showed that a random-point photographic quadrat with 64 random points was among the fastest and most objective method tested and was equally accurate, sensitive and repeatable compared to the others. Even with a unique observer, results tended to be more repeatable with RQR64 although the difference was not significant. Only 3 min were necessary for one analysis with a simulated quadrat. Moreover, with RQR64 all species with percent covers superior to 4% were detected (missed species presented maximal percent covers of 3.17% with quadrat A and 3.25% with quadrat B). According to our criteria described in the Introduction, RQR64 could be a good method for the study of coralligenous assemblages.

4.2. Random-point photographic quadrat with 64 random points applied at two field sites detects a depth gradient in species assemblages

RQR64 was easily applied to field work with 70 taxa being identifiable. Our results highlighted another important point for further sampling campaigns: 30 ($=7.5\ m^2$) or 40 ($=10\ m^2$) photos per transect produced similar species assemblage results. Thirty photos instead of 40 represented a minimum of 32 min of saved time (considering the time counted with simulated quadrats) per transect with the computer (47 min counting preparation and analysis) and 7.5 min in mean underwater. For a 30-minute dive at −60 m, 7 min was saved underwater which reduces the time spent underwater by 25 min (including decompression stages according to MN90 tables with AIR dives) and thus considerably decreases accident risks for SCUBA divers. From these photos, species assemblages were analyzed and even with our small data set, we highlighted a clear depth pattern in coralligenous assemblages. In the Mediterranean Sea, vertical distributions of subtidal assemblages figures among the most widely documented pattern; related to light, water movement, sedimentation, temperature and nutrients, depth is the major factor influencing the distribution of marine organisms (Balata and Piazzi, 2008; Balata et al., 2005; Garrabou et al., 2002; Pérès and Picard, 1964; Piazzi and Balata, 2011; Virgilio et al., 2006). After algae, Porifera generally figured among the most abundant organisms above −50 m within coralligenous assemblages (Ballesteros, 2006; Ballesteros et al., 2009; Ferdeghini et al., 2000). When *Lithophyllum* sp. and *Mesophyllum* sp. were the most dominant species at −50 m, our study showed the deepest assemblages to be dominated by Porifera (*A. cavernicola* at −60 m and *C. tailliezi* at −70 m). As the depth increased, relative cover changed (more than the number of species) and coralline algae became less and less abundant, benefiting Porifora. *A. cavernicola* was already present at −50 m at Banc des vieilles while absent above −60 m in Chrétienne. On the contrary, *C. tailliezi* appeared at −60 m at both sites. Comparison with previous studies is difficult as deep-sea coralligenous studies are scarce. ROV observations (down to −160 m) off the Spanish Mediterranean coast showed that large coralligenous concretions were more common between 80 and 120 m depth than in deeper depths (Aguilar et al., 2009). No quantitative data were available but they observed *Lithophyllum* sp. at all four sites, *A. cavernicola* at all except one, *P. clavata* and *E. cavolinii* at one site and *C. tailliezi* was never noted.

5. Conclusion

Random-point photographic quadrat sampling, with 64 random points (RQR64) was the method that cumulated the highest number of advantages. It has a good trade-off between time required, sensitivity, objectivity and accuracy, the methodology proposed here guarantees fast abundance estimation, non-destructive repeated sampling, the possibility of comparison among researchers and the permanent record of deep-sea communities. Easy to carry out and user-friendly, anybody could apply this sufficiently powerful methodology to detect multivariate patterns like depth gradient. Moreover, a new version CPCe 4.1 "coralligenous assemblage version" adapted to species identifiable in Mediterranean coralligenous assemblages is now freely available. Photos can be taken equally by ROV or divers. However, it should be avoided for total species inventory or when species richness is the variable of interest because species covering less than 4% risked to be missed. In these conditions, only total scraping with hammer and chisel allows the collection of all organisms (see for example Balata and Piazzi, 2008). This method allows the conservation of samples for further analyses (if necessary) but is highly destructive thus its application is limited and should be restricted to specialists. Visual estimations directly performed underwater benefited from recent improvements reducing the time needed for estimations to be conducted in the field (Parravicini et al., 2010) and may be preferred when one person, the specialist, is sure to evaluate all quadrates.

Acknowledgments

The study was funded by "L'Agence de l'Eau" (French Water Agency). We thank Gwenaelle Delaruelle for her help in quadrat sampling and Alison Duncan for improving English version of this manuscript. Our work is catalogued by the ISEM Steering Committee as ISEM 2012-028 (http://www.isem.cnrs.fr) **[ST]**.

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