

The influence of depth on the Mediterranean reef fish assemblage revealed by the new video on underwater scooter method

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*The new video on underwater scooter method in combination with rebreather diving techniques was introduced and applied for visual census of fish assemblages. The presently applied method facilitates deeper visual census studies below 40 m, where they have rarely been done before due to the increasing research challenges with depth. The video on underwater scooter method is also expected to be less disturbing and faster compared to swimming divers. The method was applied in visual census study of fish assemblages from 8 to 50 m depth conducted at two locations in the east Adriatic using 102 video transects. The environmental variables contributing to the variation of the reef fish community in the depth range of the infralittoral and upper circalittoral bottoms were also recorded. Thirty-one fish species were recorded on transects and 10.3% of all individuals were not identified to the species level. The average density on transects was 0.8 individuals/m², with *C. chromis*, *C. julis* and *G. auratus* being both the most abundant and the most frequent species. Three environmental variables, depth, bottom with zoocover and eastern orientation of the coast, were identified as highly significant for species occurrence. Abundance and species richness of fish assemblages showed no significant variation with depths, inclinations, orientations and sites. A significant difference in the fish assemblage structure was found among orientations, depths and inclinations with the gradient change of species composition with increasing depth and with increasing inclination.*

Key words: Underwater visual census, fish community, video transect, depth, Adriatic Sea, closed circuit rebreather

INTRODUCTION

The most common observation technique used to study reef fish assemblages is underwater visual census (UVC) with observing and recording the data *in situ* by SCUBA divers (WATSON *et al.*, 2005). The UVC method was developed in 1950' but was not applied in the Mediterranean until two decades later (BROCK, 1954; HARMELIN-VIVIEN & HARMELIN, 1975; HARMELIN-VIVIEN *et al.*, 1985). Today, UVC recorded by SCUBA divers is a standard technique used to estimate fish abundance in the field (WARD-PAIGE *et al.*, 2010). The alternative method of UVC, diver-operated video (DOV), has been increasingly used and tested in recent years for observation of infralittoral fish assemblage thanks to improvements and increased quality of video techniques (WATSON *et al.*, 2005; TESSIER *et al.*, 2005; 2013; LAM *et al.* 2006; PELLETIER *et al.*, 2011; JIND, 2012; ASSIS *et al.*, 2013; HOLMES *et al.*, 2013; WARTENBERG & BOOTH, 2015; ANDRADI-BROWN *et al.*, 2016a). The DOV is supposed to be able to reach deeper depths, removes diver effects, be cost effective and provide permanent records (CALDWELL *et al.*, 2016).

Underwater visual census methods, in general and in the Mediterranean Sea in particular, have been limited and mostly performed at shallow depth (<20 m), with the published UVC results on fish assemblages down to 30 m depth in Mediterranean not common (COLL *et al.*, 1998; GUIDETTI *et al.*, 1998; GUIDETTI *et al.*, 2002; LETOURNEUR *et al.*, 2003; PAIS *et al.*, 2004) and down to 40 m-50 m depth even more rare (FASOLA *et al.*, 1997; RENONES *et al.*, 1997; KOVAČIĆ, 2002; SOLDI & GLAVIČIĆ, 2013). The long time spent underwater to collect sufficient amount of data and need for qualified and experienced divers who are also skilled enough for identification of fish species, prevent the broad use of UVC at greater depths. In studies, using UVC on the fish assemblage composition in the Mediterranean the depth as variable was rarely assessed. A few published works assessing particularly the influence of depth were limited to 30 m depth (LETOURNEUR *et al.*, 2003; PAIS *et al.*, 2004), except RENONES *et al.* (1997) where the deepest transect reached 41 m depth.

Video-CountN transection was recommended by WARTENBERG & BOOTH (2015) as the most appropriate method for conducting DOV research and was therefore presently applied. The aims of the paper are:

1) to apply a new video on the underwater scooter method in combination with the rebreather diving techniques for visual census of fishes which is expected to be less disturbing and faster compared to swimming divers, and also facilitate visual census studies of deeper reef fish communities,

2) to identify environmental variables significantly contributing to the variation of the reef fish community at the depth range of the infralittoral and upper circalittoral bottoms by ordination statistics methods, and

3) to particularly statistically test the effects of physical and geographical factors (depth, bottom inclination, coastline orientation, locations) on the total fish abundance, species richness, and structure of the reef fish assemblage.

MATERIAL AND METHODS

Study area and sampling design

The study was carried out at two locations in the eastern Adriatic Sea on the islands of Hvar: Cape Smočiguzica and Cape Glavna (Fig. 1). Both sites are reefs with a limited width of about 200-300 m and with steep to vertical limestone bottom at depths ranging between 8 and 55 m. Both locations are similarly exposed to the prevailing weather with waves coming from the NNE, SE and SW.

The visual census was performed on 102 video transects in total from both localities at depth from 8 to 50 m depth (Table 1). The visual census was carried out by two divers, the main observer (DL) and the supporting diver (IG), using video on underwater scooter (Fig. 2A). The divers used the rebreather equipment JJ-CCR (Closed circuit rebreather) and the scooter Suex Model: XJOY7. Camera was Canon EOS 5D MK II with lens Canon EF 17-40/4L USM, 17mm with a diagonal angle

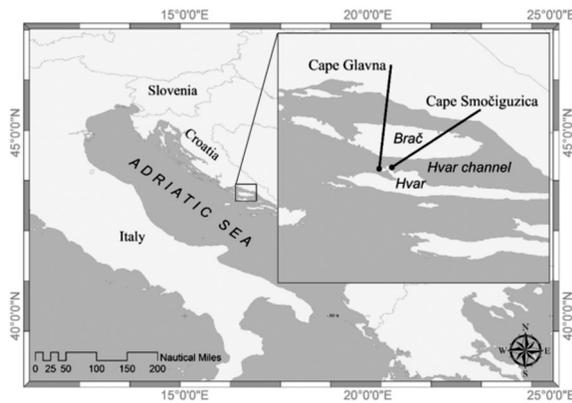


Fig. 1. Map showing studied locations at Cape Smočiğuzica and Cape Glavna

of view 104 degrees. The pretesting for finding the optimal equipment and the optimal values and conditions of shooting, and the training of the divers for the procedure took two weeks in September 2015. To avoid seasonal variability the data sampling was carried out in about one week, 2-9 June 2016. All video recordings were taken at approximately the same part of day i.e. between 11 and 16 hours. The visual census belt transect size was 25x2m. The Video-CountN transect method was adapted from WARTENBERG & BOOTH (2015).

In the pretesting process the optimal equipment, values and recording condition were investigated, including the choice of camera model, camera lenses, lighting, the best camera angle to record both epibenthic and hyperbenthic fishes, the maximum speed of the underwater scooter at which the fish are still identifiable and countable, and the distance of camera from the ground needed to adjust 2 m transect width. During the two-week pre-test phase, in addition to the finding of the optimal equipment and values and condition of shooting, the divers were trained for procedure and to maintain the distance to the bottom, to keep the correct camera angle, and to follow isobaths of the appropriate depths.

The width of the present digital transects was determined by the field of view of the camera lens (WARTENBERG & BOOTH, 2015). The conclusion from the pretesting was that the cameraman should drive an underwater scooter 1.5 m above the bottom and hold the video camera steady in front of him at an angle of 45 degrees to the

Table 1. Number of transects

Depth (m)	Number of transects
8 to 10	29
15 to 20	27
30 to 35	23
45 to 50	23
Total	102

bottom to ensure width of the transects of 2 m (Fig. 2B and 2C). However, due to the steep to vertical bottom, camera had to be angled 45 degrees to left-right and not up-down direction (Fig. 2A and 2B).

In order to avoid deploying of the transect line needed to define the transect length, thereby to reduce the diver disturbance of the bottom and to optimize the dive time (WARTENBERG & BOOTH, 2015), the transects were terminated after a fixed time, based on relationship of distance and time from the constant speed of the underwater scooter. Regarding the speed of the underwater scooter at which fishes are still identifiable, the pretesting showed that the slowest among tested underwater speeds was the best for the recognizing of fishes. The speed of a scooter with an equipment configuration (Fig. 2A) was 0.7 m/s, resulting in 36 seconds of time needed to pass 25 m transect. The visual census was performed in the conditions of absent sea current so the length of transects could be determined on the time passed. The continuing recording of transects along the same depth should be separated by intervals (ANDRADI-BROWN *et al.*, 2016a; 2016b). Therefore, the divers conducted continuous filming of several transects and the intervals between them at once in a line approximately following isobaths.

Sampling was conducted by divers in buddy team. During transecting, the primary observer (DL) in a buddy pair set the speed of underwater scooter and drove it, recording digital footage and control time for the termination of transect series. The role of supporting diver (IG) at the beginning of each transect series was to check if the horizontal visibility is good enough and if the sea current is absent not to influence the visual census and distance estimation; to establish the

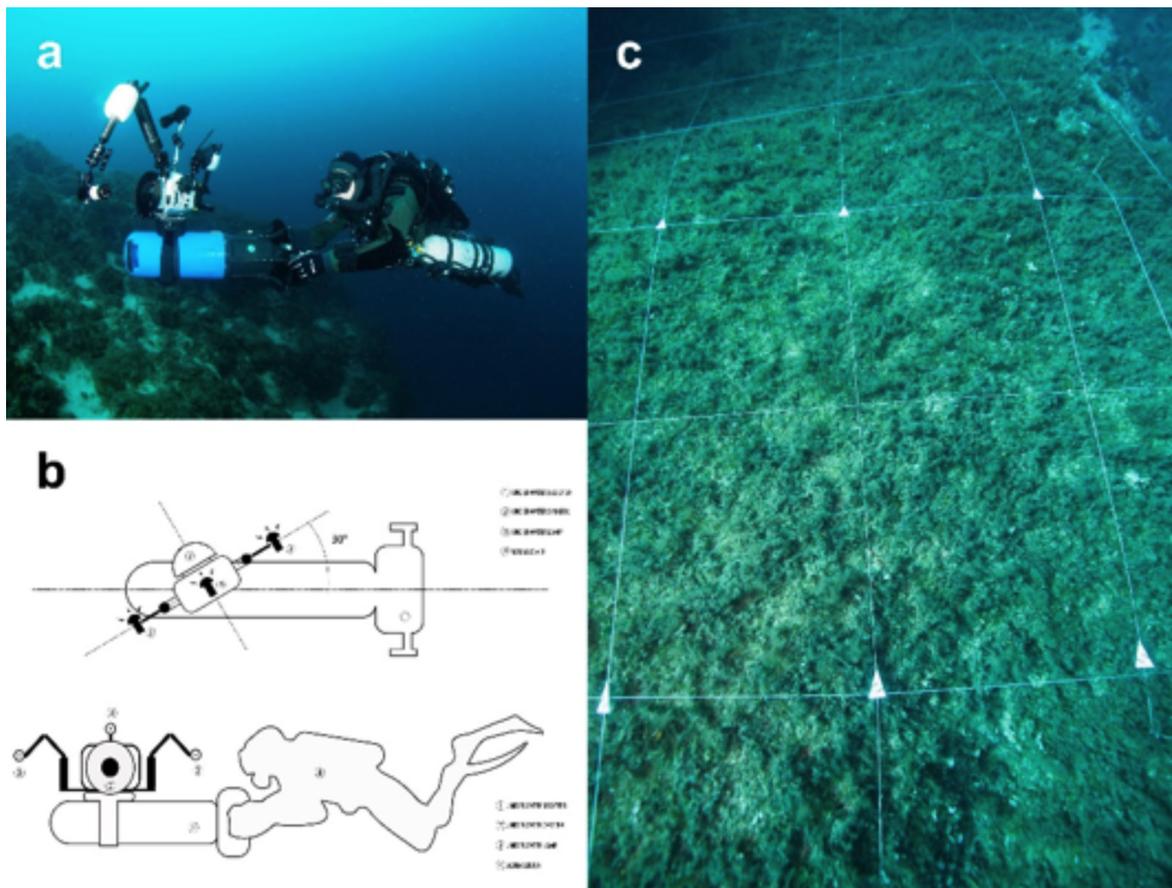


Fig. 2. a) The primary observer with full equipment, b) sketch of the mounted equipment and camera angle, c) camera view when positioned 1.5 m from the bottom and with an angle of 45 degree, grid 1 by 1 m, the view width at the closest point 2 m, widening forward out of 2 m wide marks, the view length about 5 m, visible from grid. The photos and drawing by Dani Laslo

starting point of transect series and to check at shooting beginning the distance of camera from the bottom by calibrated stick. The occurrence of a sea current was tested with a very fine bottom sediment, fine sand was dropped by second diver about 50 cm above the bottom before each survey depth, in case the sediment descends vertically to the bottom, no current is present and sampling can start. The supporting diver also recorded in situ water temperature, depth, date, time and orientation of each transect. The role of supporting diver who followed the first one during transects on the second underwater scooter was to control the procedure i.e. to control if the camera distance from the bottom and the depth i.e. following of isobaths were kept.

Media techniques and analysis

The video clips of 36 seconds from each continuous filming, which is equal to 25 m long transects (see in Study area and sampling design above), were extracted from original shooting, each with additional 14 seconds excluded as interval between transects. The initial part of video with preparation and calibration on the beginning of each shooting was also excluded. Each transect images were analyzed on a 27" screen by the fish expert (MK), using standard viewing software that enables slow view and zooming (MPC-HC 1.7.10), HPC-HC team, <http://hpc-hc.org>). Video clips were analysed on the half of the normal speed, but also using multiple pauses, additional slowing down, rewinds

and repeated playbacks whenever necessary (WARTENBERG & BOOTH, 2015). Video-CountN transecting is recommended as the most appropriate method for conducting DOVs providing highest estimates of richness and abundance, the lowest variability, the most representative diversity being also the most efficient considering time consuming (WARTENBERG & BOOTH, 2015). The CountN analysis simply identifies and enumerates all individuals observed in digital transects, effectively imitating in situ slate-transect enumeration. Individuals that could not be accurately identified to the species level because they were too far away, moved through the census area too rapidly, or were obscured due to poor lighting, camera angle, individual orientation or other factors, were categorized as unidentifiable, even if it was possible to identify them to higher taxon (WARTENBERG & BOOTH, 2015). In digital CountN analyses of all transects, first check of video clip was performed to record all present species; the second check focused on search for possible presence of fish at bottom, staying still; on the third check number of individuals of species occurring at lower densities, i.e. <10 individuals per transect, were counted simultaneously. The species that occurred at higher abundances, i.e. >10 individuals per transect were counted in separate checks/counts for each species. Counts from digital transects were corroborated by reanalyzing the media. It was assumed that if two counts for a particular transect were sufficiently similar, then those counts would have been acceptably close to the true count generated by the technique. Re-analysis was conducted 2 weeks after the original counts were completed (WARTENBERG & BOOTH, 2015). Percentage agreement was applied to the species observed (presence/absence) and to species-specific abundance for each digital transect-type analysis technique combination (WARTENBERG & BOOTH, 2015). Minimum agreement in species presence/absence was set to 100% because if a species is observed during initial analysis it should be observed in all subsequent reviews. Minimum species-specific abundance agreement was set to 100% for species observed with <10 individuals per transect, and to 90% for

species observed with >10 individuals per transect. A 10% difference in counts was considered acceptable due to the challenges of counting abundant or shoaling species in motion. When single species abundances were between 90% and 100% agreement, the mean of the two abundance estimates from the two stages of analysis was accepted as the final value. When more than one reanalysis was necessary, the percentage agreement rule was applied to the two last rounds of analysis where the minimum 90% agreement was reached. The same percentage rules were also applied to fishes categorized as unidentifiable thereby corroborating counts of all observed individuals. In addition to abundance of each recorded fish species on each transects, the supplementary habitat variables were also estimated from each digital transects in the separate check of video for each habitat variable after the fish counts: bottom inclination, bottom substrate and biocover. The variable categories were modified from KOVAČIĆ *et al.* (2012). The samples were taken at four distinct, non-contacted depth ranges and “Depth” variable was accordingly divided: 8-10 m, 15-20 m, 30-35 m and 45-50 m depth. “Inclination” as three estimated classes: gentle to moderate (<30°), steep (30° – 60°) and very steep to vertical (>60°). “Bottom substrate” as four types of different estimated particle size and three kinds of bedrock surfaces (sand, gravel <60 mm, cobbles <200 mm, boulders >200 mm, bedrock with more or less smooth surface, bedrock with crevices and cavities, high relief bedrock composed of ridges and trenches). “Biocover” as six types (no biocover, phanerogams, long thallus algae, short thallus algae, calcareous algae, zoocover). Each of the categories of “Bottom substrate” and “Biocover” present in particular sample was expressed as estimated percentage (10%, 20% ...100%) of the total surface in that sample. “Orientation” of transect was general orientation parallel to the coast as North, East and West, missing South orientation due to the coast orientation at localities. “Depth”, “Inclination”, “Orientation” therefore can be used as factors with levels, contrary to the recorded estimated percentages of bottom substrate and of the percentages of biocover types.

Table 2. The total number of recorded individuals for species and the frequency of species occurrence at the transects ($n = 102$). The number of fish is a sum on all transects. Some transects values were with decimal fractions, being the average of two counts (see Media techniques and analysis in Material and methods), with the rounding done only on the final numbers presented here

Species	Abbreviation	Total number of recorded individuals	Species frequency of occurrence
<i>Boops boops</i> (Linnaeus, 1758)	Bo bo	2	2.0%
<i>Chromis chromis</i> (Linnaeus, 1758)	Ch ch	2667	85.3%
<i>Coris julis</i> (Linnaeus, 1758)	Co ju	318	72.5%
<i>Diplodus annularis</i> (Linnaeus, 1758)	Di an	17	9.8%
<i>Diplodus puntazzo</i> (Cetti, 1777)	Di pu	1	1.0%
<i>Diplodus sargus</i> (Linnaeus, 1758)	Di sa	6	2.0%
<i>Diplodus vulgaris</i> (Geoffroy St. Hilaire, 1817)	Di vu	113	35.3%
<i>Gobius auratus</i> (Risso, 1810)	Go au	321	55.9%
<i>Gobius geniporus</i> (Valenciennes, 1837)	Go ge	1	1.0%
<i>Gobius vittatus</i> (Vinciguerra, 1883)	Go vi	9	2.9%
<i>Labrus mixtus</i> (Linnaeus, 1758)	La mi	2	2.0%
<i>Mullus surmuletus</i> (Linnaeus, 1758)	Mu su	2	1.0%
<i>Oblada melanura</i> (Linnaeus, 1758)	Ob me	2	1.0%
<i>Parablennius rouxi</i> (Cocco, 1833)	Pa ro	13	9.8%
<i>Phycis phycis</i> (Linnaeus, 1766)	Ph ph	4	2.0%
<i>Sarpa salpa</i> (Linnaeus, 1758)	Sa sa	1	1.0%
<i>Scorpaena porcus</i> Linnaeus, 1758	Sc po	3	2.9%
<i>Serranus hepatus</i> (Linnaeus, 1758)	Se he	1	1.0%
<i>Serranus scriba</i> (Linnaeus, 1758)	Se sc	43	23.5%
<i>Serranus cabrilla</i> (Linnaeus, 1758)	Se ca	13	12.7%
<i>Spicara smaris</i> (Linnaeus, 1758)	Sp sm	74	4.9%
<i>Spicara flexuosa</i> Rafinesque, 1810	Sp fl	3	2.0%
<i>Spondylisoma cantharus</i> (Linnaeus, 1758)	Sp ca	5	3.9%
<i>Symphodus doderleini</i> (Jordan, 1890)	Sy do	2	2.0%
<i>Symphodus mediterraneus</i> (Linnaeus, 1758)	Sy med	13	9.9%
<i>Symphodus melanocercus</i> (Risso, 1810)	Sy mel	3	2.9%
<i>Symphodus ocellatus</i> (Linnaeus, 1758)	Sy oc	3	2.0%
<i>Symphodus tinca</i> (Linnaeus, 1758)	Sy ti	26	16.7%
<i>Thorogobius ephippiatus</i> (Lowe, 1839)	Th ep	5	3.9%
<i>Tripterygion delaisi</i> Cadenat & Blanche, 1971	Tr de	3	2.9%
<i>Tripterygion tripteronotum</i> (Risso, 1810)	Tr tr	2	2.0%

Data analysis

In order to determine the relationship between environmental variables and the fish assemblage Canonical correspondence analysis (CCA) (TER BRAAK, 1986; 1994) was carried out using CANOCO software (TER BRAAK, 1992). All fish species recorded on the transects were used for analysis (Table 2). The species abundance were $\log(x+1)$ transformed to normalize the distribution. To reveal patterns of species response curves in relation to environmental gradients, data sets were first analyzed with Detrended Correspondence Analysis (DCA). The first axis gradient length measured in turnover (or SD units) indicated nonlinear or unimodal responses, therefore a Canonical Correspondence Analysis (CCA) was chosen as a constrained ordination method.

Among the 19 possible environmental variables included in the field recording sheet (in CCA factors with levels being just qualitative categories were decomposed and each level treated as separate environmental variables), 14 variables were actually recorded during fieldwork (Table 3).

Locality was introduced into the model as co-variable to partial out possible confounding effects varying between locations using partial constrained analysis. The first CCA was performed with all actually recorded environmental variables and the forward selection procedure was then used to extract the variables incorporated in the final model. In order to avoid inflated significance values, redundant predictor variables, i.e. environmental variables showing high multicollinearity ($VIF > 10$), were removed

Table 3. Variation explained by 14 and by 11 environmental variables. Marginal effect ($\lambda 1$) of environmental variable refers to eigenvalues fit if the corresponding variable was the only environmental variable in the model. The conditional effect (λA) refers to the additional amount of explained variance in species distribution by the correspondent variable when selected by automatic forward selection. The obtained statistics of the Monte Carlo permutation test under the full model with 999 random permutations (P - value and F) are reported for each variable. Significant conditional effect in models with 14 and with 11 environmental variables ($P \leq 0.05$) in bold

Variable	Marginal	Conditional Effects after forward selection						
	Effects	14 Environmental variables				11 Environmental variables		
	$\lambda 1$	λA	P	F	λA	P	F	
Depth	0.15	0.15	0.002	4.21	0.15	0.002	4.21	
Zoocover	0.13	0.1	0.002	2.91	0.11	0.002	2.86	
Calcareous algae	0.12	0.07	0.016	1.96	0.07	0.016	1.93	
High relief bedrock composed of ridges and trenches	0.1	0.11	0.014	2.95	n/a	n/a	n/a	
Short thallus algae	0.1	0.03	0.554	0.77	0.02	0.598	0.69	
Inclination	0.1	0.06	0.008	1.82	0.05	0.046	1.59	
East	0.09	0.09	0.002	2.56	0.09	0.002	2.5	
Sand	0.08	0.05	0.224	1.31	0.06	0.07	1.69	
Long thallus algae	0.08	0.07	0.018	2.08	0.07	0.022	2.08	
West	0.07	n/a	n/a	n/a	n/a	n/a		
No biocover	0.07	0.02	0.9	0.62	0.02	0.94	0.57	
Bedrock with crevices and cavities	0.05	0.03	0.392	0.9	0.07	0.012	2.06	
Bedrock with more or less smooth surface	0.05	0.02	0.844	0.69	0.05	0.046	1.52	
North	0.03	0.04	0.22	1.18	n/a	n/a	n/a	

from the model resulting in the final model with 11 environmental variables. The significance of the canonical axes and of the environmental variables was determined using Monte Carlo permutation tests (with 999 random permutations). The CCA was run with a focus on inter-species distances and the biplot scaling option.

The ANOVA assumptions were not met in the data for the comparisons of abundance (the total number of all individuals) and species richness (total number of recorded species) at different depths, inclinations, orientations and sites. Therefore the changes in abundance and richness among depths (8-10 m, 15-20 m, 30-35 m, 45-50 m), inclinations (<30°, 30° – 60°, >60°), orientations (N, E, W) and between two sampling sites (Cape Glavna, Cape Smočiguzica) were each examined with one-way Permutational multivariate Analysis of variance (PERMANOVA) (ANDERSON, 2001) with 999 permutation on untransformed data using Euclidian distances.

To address the possible differences in fish assemblage structures among depths, inclinations, orientations and between sampling sites we used one-way PERMANOVA, for each factor separately, with 999 permutation on $\log(x+1)$ transformed data using Bray-Curtis similarity matrix. Relevant factors were additionally tested for interaction effect with two-way PERMANOVA. All factors were treated as fixed. To graphically visualize the difference in fish assemblage structures, nonparametric multidimensional scaling (nMDS) plots (FIELD *et al.*, 1982) were performed. In cases when one-way PERMANOVA was significant ($P < 0.05$), pairwise comparison was conducted using *pairwiseAdonis* (ARBIZU, 2019) in R. The nMDS ordination plots were run using Bray-Curtis similarity matrix. We used R statistical package and *vegan* library to run all analyses.

RESULTS

Diversity, abundance and frequency of fishes

Thirty-one fish species were recorded on the 102 transects with the total number of 3677 identified individuals (Table 2) and with additional 396 individuals (10.3% of all individuals)

unidentified to the species level for the reasons listed in Material and methods. The present method was able to detect and identify benthic fish down to the size of about 8-10 cm (*Gobius geniporus* (Valenciennes, 1837), *Thorogobius ephippiatus* (Lowe, 1839)). Below that size only strikingly colored benthic species were noticed and identified due to the limits of resolution and illumination (*Gobius auratus* Risso, *Gobius vittatus* (Vinciguerra, 1883), males of *Tripterygion delaisi* Cadenat & Blanche, 1971 and *Tripterygion tripteronotum* (Risso, 1810)). The average number of all individuals per transect was 39.9, resulting in average density of 0.8 individuals/m². *Chromis chromis* (Linnaeus, 1758) was the most abundant species, with 72.5% of all identified individuals belonging to this species, followed by *Gobius auratus* Risso, 1810 (8.7%), *Coris julis* (Linnaeus, 1758) (8.6%) and *Diplodus vulgaris* (Geoffroy St. Hilaire, 1817) (3.1%). None of the other species was recorded with more than hundred recorded individuals, and 20 species were recorded with less than ten individuals, which all together represented just 1.6% of all identified individuals (Table 2). The most frequent species were again the same three most abundant species: *C. chromis* (frequency of occurrence on transects 85.3%), *C. julis* (72.5%) and *G. auratus* (55.9%), four more species were more frequent than 10%, but 8 species were recorded on just two transects and 6 species on only one transect (Table 2). The most diverse family was Sparidae (9 species) followed by Labridae (7 species).

Important habitat variables structuring the fish assemblage at the infralittoral and upper circalittoral rocky and mixed bottoms

The first partial CCA was performed with 14 environmental variables on all 31 species as response variables (Fig. 3A). The Monte Carlo permutation test was statistically significant on the first axis and also on all canonical axes (Table 3). The cumulative percentage of variance in the response data explained by the first four constrained axes was 14.2%, and the first four axes explained 64.1% of variation

in the response data explained by explanatory variables *i.e.* of the variation of fitted response values (Table 3). The model with 11 environmental variables, after the forward selection procedure excluded redundant variables (North, West, High relief bedrock composed of ridges and trenches), showed slightly lower cumulative explained variation (13.7%) and slightly higher cumulative fitted explained variation (68.0%) than the model with 14 environmental variables (Fig. 3B, Tables 4 and 5).

Canonical eigenvalues are also slightly lower in the model with 11 environmental variables (Tables 4 and 5). The forward selection identified three highly significant environmental variables: Depth, Zoocover and East ($P=0.002\%$), and, in the model with 11 environmental variables, the five other significant variables: Inclination, Bedrock with crevices and cavities, Bedrock with more or less smooth surface, Long thallus and Calcareous algae (Table 3). Among highly significant variables the first axis was positively correlated with Depth and Zoocover and negatively correlated with East, while a further positive correlation is detectable also with other three significant variables (Inclination, Bedrock with crevices and cavities, Calcareous algae) (Fig. 3, Table 3). The second axis correlate, among significant environmental variables, with Long thallus algae. The projections of species points of *Phycis phycis* (Linnaeus, 1766), *Boops boops* (Linnaeus, 1758), *Labrus mixtus* Linnaeus, 1758 and *T. ephippiatus* onto highly significant environmental variables Depth and Zoocover, but also onto significant Inclination, Bedrock with crevices and cavities and Calcareous algae, show optima of these species at high values of these environmental variables *i.e.* at deep, steep bedrock with crevices and cavities covered with sedentary animals and calcareous algae, contrary to species negatively correlated to these variables: *G. geniporus*, *Mullus surmuletus* (Linnaeus, 1758), *Serranus scriba* (Linnaeus, 1758), *T. tripteronotum*. The projections of species points of several wrasses (*Coris julis* (Linnaeus, 1758), *Symphodus doderleini* Jordan, 1890, *S. mediterraneus* (Linnaeus, 1758), *S. ocellatus* (Linnaeus, 1758)), and also of

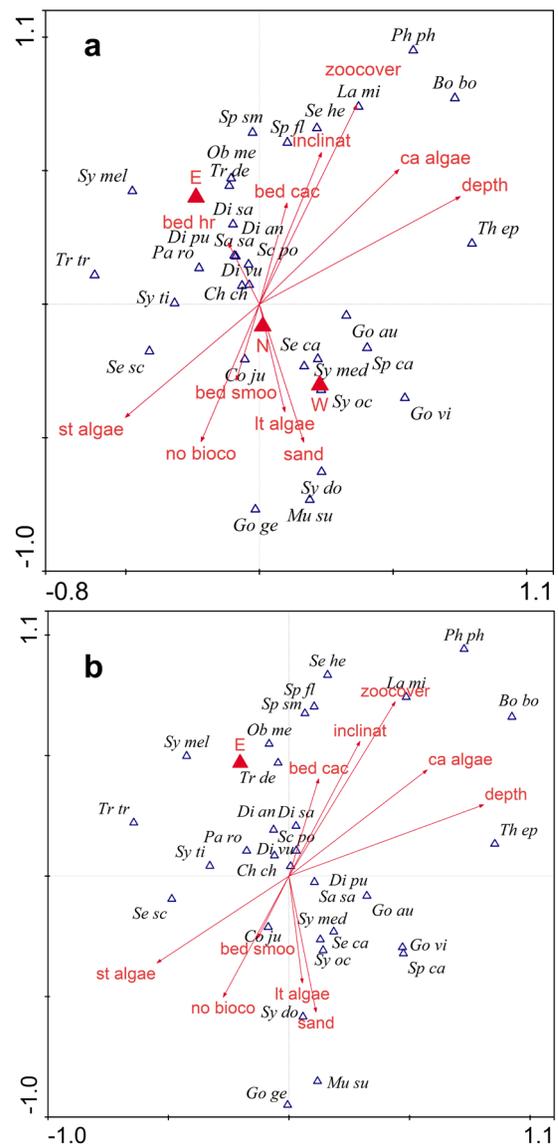


Fig. 3. CCA species-environment biplot for all 14 environmental variables (A) and final model with 11 environmental variables (B) (biplot scaling). Quantitative environmental variables are represented by arrows and nominal variable is indicated by a triangle, both red and with abbreviations in regular font. The abbreviations for explanatory variables: depth - Depth, E - East, W - West, N - North, inclinac - Inclination, sand - Sand, bed smoo - Bedrock with more or less smooth surface, bed cac - Bedrock with crevices and cavities, bed hr - High relief bedrock composed of ridges and trenches, no bioco - No biocover, lt algae - Long thallus algae, st algae - Short thallus algae, ca algae - Calcareous algae, zoocover - Zoocover. Hollow triangles and abbreviations in italics represent fish species. The abbreviations for fish species are explained in Table 2.

Table 4. Outcome of CCA, using 14 environmental variables. Significance of environmental variables are reported in Table 3

Total inertia =	3.855				
Sum of canonical eigenvalues =	0.837	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues		0.182	0.151	0.123	0.081
Species–environment (S–E) correlations		0.782	0.741	0.715	0.629
Cumulative % variance of species data		4.8	8.8	12.0	14.2
Cumulative % variance of S–E relation		21.8	39.8	54.4	64.1
Monte Carlo test (P-value, 999 permutations)		0.002	P (All canonical axes) = 0.016		

Table 5. Outcome of CCA, using 11 environmental variables. Significance of environmental variables are reported in Table 3

Total inertia =	3.855				
Sum of canonical eigenvalues =	0.764	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues		0.177	0.146	0.118	0.078
Species–environment (S–E) correlations		0.779	0.740	0.700	0.600
Cumulative % variance of species data		4.7	8.5	11.6	13.7
Cumulative % variance of S–E relation		23.2	42.3	57.8	68.0
Monte Carlo test (P-value, 999 permutations)		0.004	P (All canonical axes) = 0.014		

Spondyliosoma cantharus (Linnaeus, 1758) and *Serranus cabrilla* (Linnaeus, 1758) on significant environmental variable Long thallus algae show positive correlation of these species to the habitat with long thallus algae, while the similar biplot positions of *G. geniporus*, *M. surmuletus* and also *G. vittatus* is probably more related to another quantitative predictor, Sand, which is highly overlapped with Long thallus algae, and had large but not significant effect. The deep bottom species *P. phycis* and *L. mixtus*, benthopelagic species *Spicara flexuosa* Rafinesque, 1810, *S. smarís* (Linnaeus, 1758) and *Oblada melanura* (Linnaeus, 1758), and also *Serranus hepatus* (Linnaeus, 1758) avoided of Long thallus algae habitat.

The variation of the fish abundance, species richness and fish assemblage

structure with depths, inclinations, orientations and sites

PERMANOVA for the differences in total fish abundance and the species richness showed no significant differences with depths, inclinations, orientations and sites (Table 6).

However, the PERMANOVA test on the fish assemblage structure indicates the significant difference in fish assemblage structures among different orientations, depths and inclinations, while sites didn't have significant influence on the fish assemblage composition (Table 7). The post hoc test reveals that the fish assemblage at shallow depth, 8-10 m, is different from the fish assemblages at all other depths, but also that the fish assemblage composition at the depth 15-20 m is again significantly different from the largest depth, 45-50 m (Table 7). The pairwise PERMANOVA also indicated the significant difference in fish assemblage between extreme inclinations i.e. <30° and >60° (Table 7). The results of the pairwise comparison for depths

Table 6. Results of one-way PERMANOVA tests comparing total fish abundance and species richness of fish assemblages according to four factors: Depth (8-10 m, 15-20 m, 30-35 m, 45-50 m), Inclination (<30°, 30°–60°, >60°), Orientation (North, East, West) and Sites (Cape Glavna, Cape Smočigizica). * $P < 0.05$; ** $P < 0.001$; n.s.=not significant. Analyses were conducted on untransformed data using Euclidian distances

Source of variation	df	ABUNDANCE			SPECIES RICHNESS		
		Pseudo F	P (perm)		Pseudo F	P (perm)	
Site	1	0.014	0.923	n.s.	0.625	0.426	n.s.
Orientation	2	1.425	0.259	n.s.	0.642	0.544	n.s.
Depth	3	0.956	0.407	n.s.	0.465	0.725	n.s.
Inclination	2	1.758	0.182	n.s.	2.394	0.084	n.s.

and for inclinations therefore imply the gradient changes in species composition with the increasing depth and with the increasing inclination. In addition, it looks like the intensity of changes in species composition was larger in shallow water since 8-10 m and 15-20 m were the only neigh-

Table 7. Results of PERMANOVA tests comparing fish assemblage structure according to four factors: Depth (8-10 m, 15-20 m, 30-35 m, 45-50 m), Inclination (<30°, 30°–60°, >60°), Orientation (North, East, West) and Sites (Cape Glavna, Cape Smočigizica). * $P < 0.05$; ** $P < 0.001$; n.s.=not significant. Results of pairwise comparison are reported only for factors with more than two levels having significant ($P < 0.05$) difference. All data were $\log(x+1)$ transformed

Source of variation	df	Pseudo F	P (perm)	
Site	1	0.812	0.537	n.s.
Orientation	2	3.369	0.03	*
Depth	3	15.589	<0.001	**
Inclination	2	9.574	<0.001	**
Pairwise comparison				
Orientation				
E vs N		2.58	0.06	
E vs W		4.10	0.006	*
N vs W		3.36	0.022	*
Depth (m)				
8-10 vs 15-20		12.05	<0.001	**
8-10 vs 30-35		15.952	<0.001	**
8-10 vs 45-50		19.464	<0.001	**
15-20 vs 45-50		3.421	0.025	*
Inclination				
<30° vs >60°		9.31	<0.001	**

boring depth ranges with significantly different fish assemblage composition. The nMDS plots illustrated the differentiation of fish assemblage species composition among depths (Fig. 4A), inclinations (Fig. 4B) and orientations (Fig. 4C) found by the PERMANOVA test, while there was no separation on the nMDS ordination plot between fish assemblage species composition at different sites, matching again the PERMANOVA results (Fig. 4D). Even the significant differences established from the pairwise comparison are visible on the nMDS plots as the larger distances between centroids of those levels on the plots (Table 7, Fig. 4A and 4B). There was also the significant interaction between site and depth, site and orientation and orientation and depth found by the two-way PERMANOVA (Table 8), meaning that the effect of depth varied between sites and among different orientations and the effect of orientation varied between sites.

Table 8. Results of two-way PERMANOVA tests comparing fish assemblage structure. * $P < 0.05$; ** $P < 0.001$; n.s.=not significant

Source of variation	df	Pseudo F	P (perm)	
Site x Depth	3	2.376	0.01	*
Site x Orientation	2	3.660	0.02	*
Site x Inclination	5	0.221	0.956	n.s.
Depth x Inclination	5	0.925	0.522	n.s.
Depth x Orientation	5	2.020	0.015	*

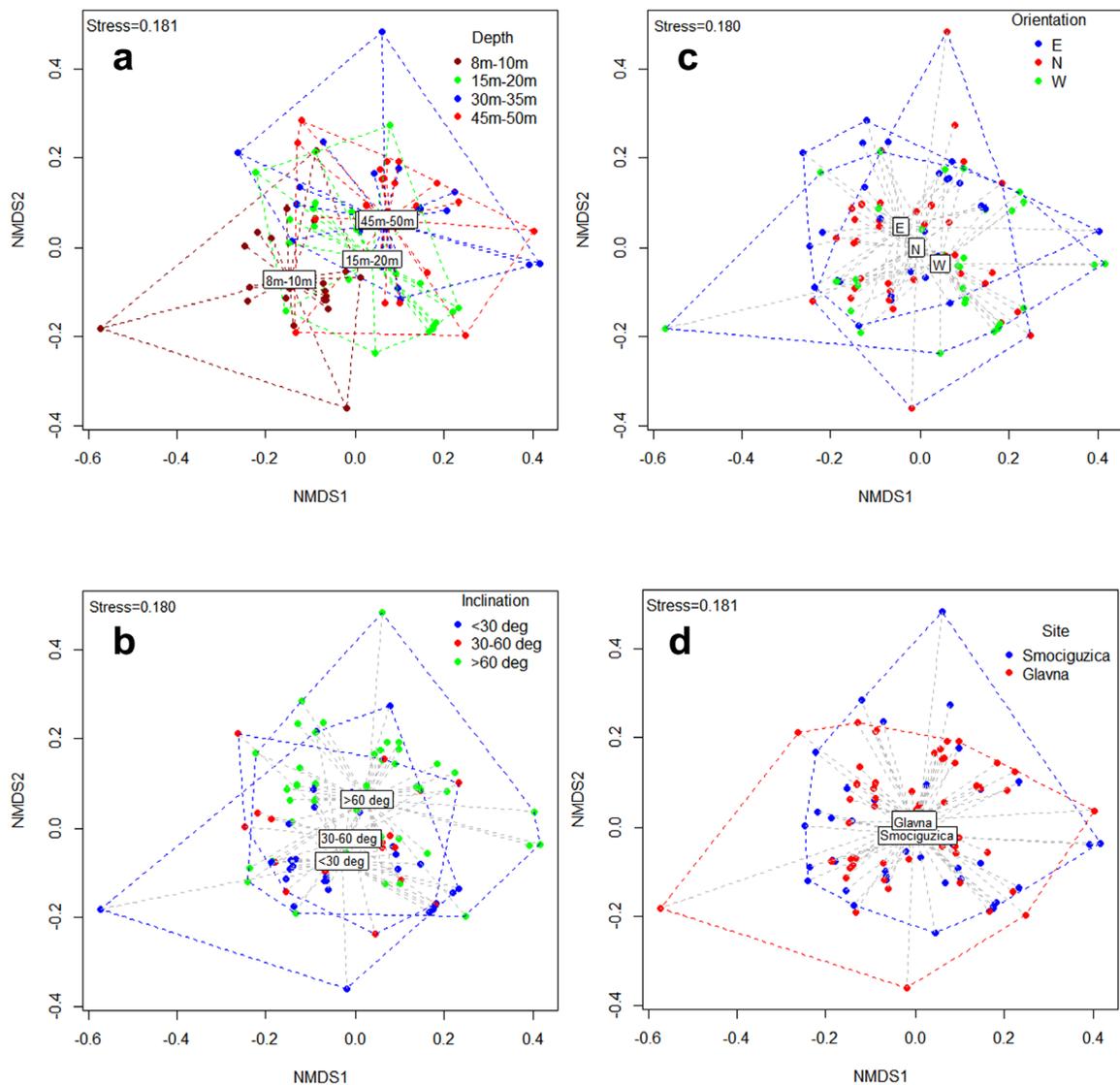


Fig. 4. Non-metric multi-dimensional scaling (nMDS) ordination plot of individual transects comparing (a) depth: 8-10 m (brown circles), 15-20 m (green circles), 30-35 m (blue circles), 45-50 m (red circles); (b) inclination: <math><30^{\circ}</math> (blue circles),

DISCUSSION

The present method showed to be limited to detect and identify benthic fish below a size of about 8-10 cm, except for the conspicuously colored fish, due to the limits of resolution and illumination. Similar to other DOV studies and to UVC methods in general, in the present study we were also not able to detect cryptobenthic fishes (KOVAČIĆ *et al.*, 2012), which are by definition, hidden in bottom space and out of reach

by the visual methods. Therefore, the presently described fish assemblages are incomplete lacking these parts of the fish assemblage. This general limitation of visual methods prevents the visual census from being able to describe the entire fish assemblage of the bottom habitats.

In comparison with other visual censuses carried out, which also examined the influence of depth on the fish assemblage composition in the Mediterranean, the present data showed lower total diversity (31 species recorded over

48 species in RENONES *et al.* (1997); 62 species in LETOURNEUR *et al.* (2003); and 47 species in PAIS *et al.* (2004)). However, those studies were conducted on a more variable range of habitats compared to this study which was limited to underwater cliffs. Interestingly, the most abundant fish species in all studies was *C. chromis*, becoming candidate for the most abundant fish species of the non-sandy infralittoral bottoms of the northern Mediterranean coasts. Furthermore, part of the *C. chromis* population is known to hide in cryptic bottom spaces even during daylight (KOVAČIĆ *et al.*, 2012), so the total abundance of *C. chromis* is probably even larger than the abundance of the exposed visible individuals established by the present visual census. Unfortunately, in RENONES *et al.* (1997), LETOURNEUR *et al.* (2003) and PAIS *et al.* (2004) no data on average density of the whole sample was available for comparison.

In contrast to the other significant environmental variables that influence species composition, e.g. depth and inclination, bottom structure (bedrock with crevices and cavities or with more or less smooth surface) and biocover (zoocover, calcareous algae or long thallus algae), the high significance of eastern position for the fish assemblage structure is the only puzzling result among the explanatory variables. It is positively correlated with the second axis, and although the eastern position could be expected to have better sunlight conditions, its centroid position on the biplot (Fig. 3) is opposite to environmental variables that depend on sunlight e.g. long thallus algae. During the fieldwork, no prevailing influence of any particular depth, inclination, bottom structure or biocover was observed on the eastern transects, which is confirmed from the biplot where almost no other explanatory variables are directed near the eastern position centroid (Fig. 3). Another significant environmental variables in this study have clearly structured the fish assemblage and the results showed that the fish assemblages of deep, steep bedrock with crevices and cavities covered with sedentary animals and calcareous algae (*P. phycis*, *L. mixtus*, *T. ephippiatus*) or of the long thallus algae bottoms (Labridae, *S. cantharus*) are composed of

species which presence there match our general knowledge of the species habitat preferences (JARDAS, 1996).

The abundance and species richness of the fish assemblages did not show significant variation with depths, inclinations, orientations and sites. However, in contrast to the results of previous studies (RENONES *et al.*, 1997; LETOURNEUR *et al.*, 2003; PAIS *et al.*, 2004), we were able to find, by Permutational multivariate analysis of variance, the significant difference in fish assemblage among different depths, with the shallowest depth, 8-10 m, having the most unique composition and with the gradient change of species composition with increasing depth down to the deepest transects at 45-50 m depth. This is consistent with the results of our Canonical correspondence analysis, which reveals depth as a highly significant environmental variable that influences species composition. LETOURNEUR *et al.* (2003), from the Canonical correspondence analysis, showed that depth in itself was not a significant factor explaining fish species patterns from 0 to 30 m depth and that the most significant factors were bottom substrate (sand) and biocover (macroalgae and *Posidonia oceanica*). The research carried out by PAIS *et al.* (2004) at 0 to 30 m depth was limited by the method to reveal significant differences in fish assemblage at different depths, as the depth as a factor was only tested by ANOVA for density of each fish species, with only two species, *C. chromis* and *S. maena*, showing significantly different density with depth. Finally, RENONES *et al.* (1997) showed by cluster analysis the greatest difference in similarity between their deeper stations at 41 m depth and all other stations and by correspondence analysis they found difference among block stations, vertical cliff stations and again for two deep stations at 41 m depth. The presently found depth importance for the fish assemblage structure was probably not established in previous three works probably for several reasons:

- 1) they did not focus any of their analyses primarily on depth as an environmental variable with appropriate hypothesis testing tool,
- 2) they mostly limited their analysis to the

infralittoral zone while the present research was extended to the upper circalittoral,

3) in contrast to the more homogenous bottoms along the depth range in the present research, the variability of the involved bottom types in earlier researches could obscure the differences by depth in previous studies.

The present study did not compare the results of the video on underwater scooter method with the UVC and or with the DOV data. The simultaneous comparison, like in comparisons between UVC and DOV results (e.g. TESSIER *et al.*, 2013), would be hard to achieve considering the speed of the underwater scooter against the diver swim. Slate recorded UVC requires a long underwater time to collect sufficient data and also requires divers who are experienced ichthyologists or others trained in identifying species and in some cases estimating individual fish sizes. However, it is well known that the slate recorded UVC gather better information as method since the video census is less accurate than standard UVC for determining species richness and video census also underestimates abundance, especially for low values (TESSIER *et al.*, 2005). Anyway, the present video on underwater scooter method in combination with rebreather diving techniques could have two advantages, both over standard UVC and over DOV studies of fish assemblages, the decreased disturbance of fish assemblages and the greater depth that could be reached. Without pre-constructed transects, with the short time of smooth passage of the transect areas with the underwater scooter and with the noiseless breathing of the mixed-gas closed-circuit rebreather the disturbance of fish assemblages should be greatly reduced compared to standard UVC and DOV methods. LINDFIELD *et al.* (2014) already showed that the use of the mixed-gas closed-circuit rebreather for fish surveys clearly minimizes behavioral biases associated with fish avoiding open-circuit SCUBA divers. The video on underwater scooter method in combination with the rebreather diving techniques also allows to reach greater depth, being faster with the underwater scooter and staying deeper and longer, thanks to the mixed gas closed circuit rebreather system, so

the deeper underwater visual census could be the niche for the application of the new presented method.

Over the last thirty years, the faster alternative to the UVC, using underwater video techniques, has been introduced and used for assessing fish assemblages (GREENE & ALEVISION, 1989, FRANCOUR *et al.*, 1999; WILLIS & BABCOCK, 2000; HARVEY *et al.*, 2001). The ever increasing resolution and performance in low light conditions of each new generation of cameras and their sensors make underwater video census increasingly promising method. Over the last two decades, diving techniques with advanced dive equipment and different gas mixtures have been used to allow safe excursions to depths of 50–150 m, which exceeds the depths that can be safely reached by conventional SCUBA (PARRISH & PYLE, 2002). Despite these advances in SCUBA diving, few results have generally been published on the visual census of fish assemblages at depths of 50 m or deeper (BROKOVICH *et al.*, 2008 to 65 m depth; BEJARANO *et al.*, 2010; 2014 to 70 and 85 m depth; PINHEIRO *et al.*, 2015 to 130 m depth), probably due to the increased logistical challenges associated with depth even today (ANDRADI-BROWN *et al.*, 2016a; 2016b). Two diving techniques make it possible to approach to these depths. The first one is open-circuit trimix SCUBA equipment, which has rarely been used for studies of fishes, with the single example of collecting fishes in Mediterranean (GLAVIČIĆ & KOVAČIĆ, 2016). However, gas spending of this open-circuit rig limited the effective bottom times at greater depths to no more than 12-15 minutes (PYLE, 1999) so its use is limited to short tasks. The second technique is mixed gas closed circuit rebreather (CCR) technology which is used even more rarely applied in studies of fishes than open-circuit trimix SCUBA technology (PARRISH & PYLE, 2002; BEJARANO *et al.*, 2014; PINHEIRO *et al.*, 2015). Compared to open-circuit trimix SCUBA equipment, CCR has much higher autonomy underwater, decreases the risk of decompression sickness, reduces overall gas requirements, increases overall safety, and is bubble-free (PINHEIRO *et al.*, 2015). These properties make it more promising for use in the visual

census of deep bottom fish assemblages. However, CCR has serious limitations in terms of the price of equipment and the cost of fieldwork, as well as the need for highly skilled and trained CCR divers who must also be at the same time experts in fish species identification. The use of underwater video technique can overcome the later limitation by separating the CCR diving skills from the fish species identification knowledge. Rebreather diving techniques combined with the less time consuming visual census

method thanks to the higher speed of the underwater scooter and the high quality video camera could make visual census studies below 40 m, even down to 100 m depths, a standard method.

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Utjecaj dubine na zajednicu riba stjenovitog dna u Mediteranu istraženu novom metodom videa sa podvodnog skutera

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SAŽETAK

Nova metoda videa sa podvodnog skutera uz rebreather ronilačku tehniku uvedena je i primjenjena za vizualni census zajednica riba. Ova metoda omogućuje istraživanja vizualnim censusom na dubinama većim od 40 m, na kojima se, zbog teškoća u ronjenju na većim dubinama, vizualni census rijetko izvodi. Metoda videa sa podvodnog skutera uz rebreather ronilačku tehniku također bi trebala biti manje uznemirujuća i brža u odnosu na plivajuće ronioce. Metoda je primjenjena u istraživanju vizualnim censusom na dubinama 8 do 50 m na dvije postaje na istočnom Jadranu sa 102 video transektu. Zabilježene su također varijable okoliša koje utječu na varijaciju u sastavu zajednice riba stjenovitog dna infralitorala i cirkalitorala.

Trideset i jedna vrsta riba je zabilježena, uz 10,3% primjerka koji nisu određeni do razine vrste. Prosječna gustoća riba po transektu bila je 0,8 primjeraka/m², a najčešće i najbrojnije vrste bile su *C. chromis*, *C. julis* i *G. auratus*. Tri varijable okoliša: dubina, dno sa životinjskim obraštajem i istočna orijentacija dna, bile su izrazito značajne za prisutnost vrsta. Brojnost primjeraka i bogatstvo vrsta nisu pokazale značajnu promjenjivost sa promjenama dubine, nagiba, orijentacije i postaja. Utvrđena je značajna promjena u sastavu zajednice riba sa promjenom orijentacije obale, dubine i nagiba dna na transektima. Sastav vrsta postepeno se mijenjao sa porastom dubine dna i s povećanjem nagiba dna.

Ključne riječi: podvodni vizualni census, zajednica riba, video transekt, dubina, Jadransko more, rebreather zatvorenog kruga