A quantitative assessment of the diel influence on the cryptobenthic fish assemblage of the shallow Mediterranean infralittoral zone

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Summary: Cryptobenthic fishes are an underestimated and probably important component of coastal marine ecosystems that are usually overlooked by standard methods for collecting and studying benthic fishes. Studies focusing on cryptobenthic fishes have been rare and all have been based on samples taken during daytime. The present study tested the difference in epibenthic and cryptobenthic fish composition, diel differences in cryptobenthic fish assemblage and diel shifts of infralittoral fish species between hidden and open bottom spaces. It also looked for the significant habitat variables structuring the cryptobenthic fish assemblage. The daylight, sunset and night samples of epibenthic and cryptobenthic fish assemblages were collected from 78 squares of 1 m^2 shallow water plots (0.5 to 3 m) on Brač island in the eastern Adriatic. The study recorded 27 species, among which the family Gobiidae dominated fish diversity with 14 species. Cryptobenthic specimens highly outnumbered epibenthic specimens, with a ratio of 7.5 to 1. Species composition of cryptobenthic and epibenthic fish assemblages differed significantly. No diel variation in composition, species richness or abundance of the cryptobenthic fish assemblage was detected. Occurrence frequencies in hidden and open bottom spaces of ambivalent species did not change significantly between times of day, so no dial switches between open and hidden places were apparent. In combination, these results suggest that the cryptobenthic fish assemblage has diel stability and is mostly composed of permanent inhabitants of hidden spaces with domination of miniature gobies. Significant habitat variables for species occurrence were the presence of multiple layers, bottom inclination and the presence of cobbles, while depth also had a large but not significant effect.

Keywords: cryptic fishes; diel influence; multivariate analysis; habitat utilization; benthos; littoral zone.

Una evaluación cuantitativa de la influencia diaria sobre la comunidad de peces criptobentónicos en la zona infralitoral mediterránea

Resumen: Los peces criptobentónicos están subestimados y probablemente forman un componente importante de los ecosistemas costeros debido a errores en los métodos de muestreo habituales en peces bentónicos. Los estudios sobre peces criptobentónicos son escasos y generalmente realizados durante el día. El presente trabajo analiza la diferencia en la composición de peces epibentónicos y criptobentónicos, diferencias día-noche entre especies criptobentónicas y cambios diarios de peces infralitorales entre áreas abiertas y ocultas. Asimismo, se analizan las variables del hábitat que estructuran las comunidades criptobentónicas. Se realizaron muestreos durante el día, anochecer y noche de las comunidades epibentónicas y criptobentónicas a partir de 78 cuadrados de 1 m^2, entre 0.5 y 3 m en la isla Brač (Adriático oriental). El estudio registró 27 especies, siendo dominante la familia Gobiidae con 14 especies. Los ejemplares criptobentónicos sobrepasan claramente a los epibentónicos (7.5 a 1). La composición de especies de ambas comunidades son muy diferentes. En la comunidad criptobentónica no se observaron ni variaciones diarias en su composición, ni en la riqueza o abundancia de especies. La frecuencia de especies entre zonas abiertas u ocultas no cambia según la hora del día. En conjunto, estos resultados sugieren que la comunidad criptobentónica tiene una estabilidad diaria y que está formada sobre todo por especies que viven en zonas ocultas y por pequeños góbidos. Las variables del hábitat más significativas son la inclinación del fondo y la presencia de bloques, mientras la profundidad influye, pero sin ser significativa.

Palabras clave: peces crípticos; influencia diaria; análisis multivariante; uso del hábitat; bentos; zona litoral.

INTRODUCTION

Cryptobenthic fishes most likely play an important role in coastal marine ecosystems, but large gaps of knowledge about their functionality remain (Goatley and Brandl 2017). A cryptobenthic fish is a species (or a life history stage of a fish species) whose individuals exclusively or predominantly spend their lifetime in cryptobenthic microhabitats, i.e. in the restricted living spaces underneath the bottom surface of the substrate or biocover, with a physical barrier to open spaces (according to the habitat or ecological definition of Kovačić et al. 2012). Standard methods for collecting and studying benthic fishes mostly fail to collect or record cryptobenthic fishes (Ackermann and Bellwood 2000, Willis 2001, Smith-Vaniz et al. 2006). According to the comparative study of Smith-Vaniz et al. (2006), the unrecorded biodiversity of the fish assemblage estimated by visual census was very high, as they visually recorded only 36% of 228 fish species they sampled with rotenone. It was estimated that cryptobenthic fishes (known and still undescribed species) could represent more than half of the total coral reef fish biodiversity (Brandl et al. 2018). These fishes by definition have an intimate association with the benthos and provide important food web links between the benthos and nekton communities of coral reefs, with their quantitative significance resulting from their exceptional growth rates and mortality (Goatley et al. 2016). Most quantitative ecological studies that differentially have targeted microhabitats of cryptobenthic fishes have been performed in coral reef and seagrass meadow ecosystems (Depczynski and Bellwood 2003, Ahmadia et al. 2012, Tornabene et al. 2013), a few in south temperate reefs (Prochazka 1998, Willis and Anderson 2003), and only a few in northeastern Atlantic (Beldade and Goncalves 2007) and Mediterranean warm temperate reefs (Kovačić et al. 2012, Glavičić et al. 2016, Thiriet et al. 2016). Mainly on the basis of these studies, cryptobenthic reef fishes are now considered to play a globally significant role in temperate and tropical reefs: they are numerically dominant across different cryptic microhabitats, they may serve as a key trophodynamic component between open water and seafloor habitats, and ecologically highly differentiated species assemblages evolve rapidly in different microhabitats and regions and hence may serve as sensitive environmental indicators (Goatley and Brandl 2017).

Interestingly, all aforementioned studies were based on samples taken during daytime, and virtually nothing is known about diel changes of the cryptobenthic fish assemblage. Except for highly specialized coral or sponge dwelling species, very little is known also about the autecology of most cryptobenthic fish species (Goatley and Brandl 2017).

The studies of diurnal-nocturnal activity of inshore benthic fishes started half a century ago (Hobson 1965), but crepuscular and nocturnal fish assemblages of open benthic habitats remain largely unresearched in shallow coastal environments (Azzurro et al. 2007). In the Mediterranean Sea, only a few published studies on diel variations of littoral assemblage of epibenthic and hyperbenthic fish have been performed on soft sediments down to a few metres depth by beach seine (Dulčić et al. 2004, 2005) or on rocky bottoms by visual census performed by SCUBA dives down to 3 m (Azzurro et al. 2007, 2013). Published studies clearly show diel variations of the littoral assemblage of epibenthic and hyperbenthic fish. Dulčić et al. (2004) found exclusively nocturnal and exclusively diurnal fish species and major diel differences in total fish abundance and biomass. Azzurro et al. (2007) revealed a clear-cut separation between day and night fish assemblages, with significant differences in species composition, species richness and fish abundance.

Considering the lack of any data on diel variations in cryptobenthic fish assemblages and the rarity of studies on diel shifts of other littoral benthic fishes in general, the general goal of the present study was to fill this knowledge gap, which might hide important aspects of the dynamics and relationships of coastal inshore fish assemblages. The quantitative assessment of littoral cryptobenthic fish assemblages of the shallow infralittoral (0.5-3 m depth) also complements earlier studies of littoral cryptobenthic fish assemblages performed deeper by Kovačić et al. (2012) and Glavičić et al. (2016). The present research used the cryptobenthic fish definition of Kovačić et al. (2012) based on the fish position at the bottom, so the aim was to study the fish assemblage in the hidden physical space, "inside bottom" (cryptobenthic in this work), and to compare it with the fish assemblage laying on the open bottom (epibenthic in this work). Therefore, the specimens could be cryptobenthic or epibenthic, according to the place of collection, while the species status as the sum of positions of all specimens could be exclusively or predominantly cryptobenthic, exclusively or predominantly epibenthic or ambivalent (termed epicryptobenthic by Kovačić et al. (2012)) if specimens were considerably recorded in both bottom spaces. In particular, the study was designed (1) to test whether epibenthic and cryptobenthic fish compositions differ; (2) to investigate differences in composition, species richness and abundance of cryptobenthic fish assemblages at day/night/sunset, which could be interpreted as time of day–dependent changes; (3) to test for diel shifts of infralittoral fish species between hidden and open spaces, by testing whether ambivalent species present in both hidden and open spaces exhibit significantly different epibenthic and cryptobenthic frequencies of occur-

rence at daylight, night and sunset; and (4) to identify environmental variables contributing significantly to the variation of the cryptobenthic fish assemblage in the shallow infralittoral zone (0.5-3 m depth), including also the time of day as an explanatory variable.

MATERIALS AND METHODS

Sampling location

The study was performed in the eastern Adriatic, on the south side of Brač island, about 1.5 km east of the city of Bol, in a small bay (43°15.543′N, 16°38.607′E) located in front of a tourist resort. The sea bottom at the study depths consisted of sand and various mixed rocky bottom types (bedrock, boulders, cobbles and pebbles) covered with sessile biota, including a variety of algae, sponges, sea urchins and sea cucumbers. Areas deeper than 3 m are mostly covered by *Posidonia* meadows. There are no strong currents at the location, underwater visibility is typically 20-25 m at water depths of less than 5 m, the position is daily exposed to the afternoon waves by the maestral wind, and the strongest wave action on this position is produced by an occasional sirocco wind.

Data collection

Data and specimens were collected by two SCUBA divers (IG and MK) during three surveys performed on 9-11 June 2015, 10-14 May 2016 and 10-13 October 2016. The number of squares performed in one dive varied from three to five depending on the time of day and other conditions, with two dives on average per day.

Dives were performed during daylight, sunset or night: 26 daylight, 25 sunset and 27 night bottom squares were sampled, giving the total of 78 sampled bottom squares of 1 m². The protocol for selecting squares was adopted and modified from Kovačić et al. (2012): about an equal number of squares were sampled during daylight, sunset and night and about an equal number of squares were studied at each depth in shallow water: 0.5, 1, 2 and 3 m. Underrepresented day time, depth and dominant substrate type categories at a particular moment of the fieldwork were established from the already collected data and targeted for the next dive. Therefore, in addition to daytime and depth, squares were further selected to achieve as equal as possible a representation of each dominant substrate type on each of the isobaths. To achieve this, each isobath was followed until the next not yet sufficiently sampled dominant substrate type was found, at least 10 m from the previous square (Kovačić et al. 2012). The protocol for sampling in squares was also adopted and modified from Kovačić et al., (2012): (1) visual census of the epibenthic fishes, (2) fixing of a reference frame (1×1 m) onto the bottom, (3) photography of the 1 m² square taken and stored as a reference for in situ estimations of percentages of habitat parameters "bottom substrate" and "biocover", and number by size of the habitat parameter "number of semi-caves, caves and cavities", all previously estimated in situ, (4) recording of habitat characteristics in 1 m² and temperature, (5) spraying of the anaesthetic into the square, catching escaping fishes with a handnet and waiting for about 2 min, and (6) removal of biocover and movable parts of the bottom structure and collection of anesthetised fishes. The anaesthetic used was Quinaldine, diluted 1:15 with 96% ethanol and then mixed 1:5 in 750 mL bottles with sea water (modified from Kovačić et al. 2012). Two bottles were usually used for one square with the total volume of deployed quinaldine-ethanol solution per square of about 300 mL/m². The habitat variables "bottom substrate", "number of bottom layers", "biocover" with categories match Kovačić et al. (2012) as follows: "bottom substrate" as five types of different estimated particle size (sand <2 mm, gravel <60 mm, cobbles <200 mm, boulders >200 mm, bedrock) expressed as estimated percentages (10%, 20%... 100%) of the total surface in each frame (1×1 m); "number of bottom layers", i.e. no layers, single layer, multiple layers; and "biocover" as six types (no biocover, phanerogams, long thallus algae, short thallus algae, calcareous algae, zoocover). Other variables were modified: "depth" (0.5, 1, 2 and 3 m); "inclination", as five estimated classes (gentle 0-19°=1, medium 20-59°=2, steep 60-74°=3, very steep to vertical 75-90°=4, overhanging=5); and "number of semi-caves, caves and cavities", if present, as number of each of four estimated size classes of hidden spaces in each square by entrance size (25-99 cm², 100-499 cm², 500-1999 cm², >2000 cm²). All collected specimens were killed after SCUBA dives by over-anesthetization with quinaldine and stored in 65% ethanol solution. Standard length of each fish was measured thereafter. Preliminary field species identifications of cryptobenthic specimens were later rechecked in the lab on preserved specimens.

Data analysis

Difference between epibenthic and cryptobenthic samples in species composition in total and at different times of day. Significant differences between epibenthic and cryptobenthic species compositions were tested using analysis of similarities (ANOSIM) with a Bray-Curtis dissimilarity index for the dissimilarity matrix and 999 as the number of permutations.

Differences in cryptobenthic fish assemblage species composition, total fish abundance and species richness between daylight, night and sunset samples. Similarity of species composition among studied squares was graphically represented by two-dimensional non-metric multi-dimensional scaling using the Bray-Curtis similarity matrices (Clarke 1993). Species abundance data were square-root transformed to reduce weighting given to abundant species (Clarke 1993). Significant differences between daylight, night and sunset cryptobenthic species compositions were tested with ANOSIM with a Bray-Curtis dissimilarity index for the dissimilarity matrix and 999 as the number of permutations. One-way analysis of variance (ANOVA) was used to test for significant differences in total fish abundance and species richness between

daylight, night and sunset squares. Total abundance and species richness data were square-root transformed for counts per unit data (Underwood 1997). The homogeneity of variance was tested using Cochran's test on transformed data.

Difference in epibenthic and cryptobenthic frequencies of occurrence of ambivalent species between daylight, night and sunset. Significance of differences in epibenthic and cryptobenthic frequencies of occurrence of the ambivalent species between daylight, night and sunset was tested by the Fisher exact test, considering the small size of samples (McDonald 2014). The size of the tested contingency table was limited to 2x2 to fit the Fisher exact test, so three pairs of times of day were compared separately. Since row and column totals are unconditioned, the Fisher exact test is not, strictly speaking, exact. Instead, it is somewhat conservative, having a little less statistical power (McDonald 2014). Since in ambivalent species the total sum of frequencies of two categories of both groups was less than or equal to six, the data from all ambivalent species were pooled together by category and by group, and significance was tested on frequencies summed from all species. *Millerigobius macrocephalus* (Kolombatović, 1891) and *Zebrus zebrus* (Risso, 1827) had numerous specimens found in hidden spaces and single and two specimens, respectively, found on the open bottom (Table 1). Strictly speaking, they are not ambivalent species but they were also included in the test, separately from ambivalent species (Table 1).

Influence of environmental explanatory variables and time of day as an explanatory variable on cryptobenthic fish species as response variables. Community composition of cryptobenthic fishes was analysed in order to detect explanatory variables (including both the habitat variables and the times of day) that best explain species distribution and abundance patterns. Only cryptobenthic specimens from species with four or more cryptobenthic specimens were included in the analyses (modified from Glavičić et al. 2016). Therefore, only the 13 most common species were used for analysis (Table 1). Following the semantic-based approach for transformation (Šmilauer and Lepš 2014), the data were log(x+1)-transformed because we asked for the amount of change of species abundance by unit change of environmental variables. Among the 20 possible environmental variables included in the field recording sheet, 18 variables were actually recorded during fieldwork, of which one (phanerogams) with presence in just two cases was discarded; hence 17 variables were finally included in the analysis (Table 2). To reveal patterns of species response curves in relation to environmental gradients, data sets were first analysed with detrended correspondence analysis. The first axis gradient length measured in turnover (or SD units) was 4.46, indicating strong nonlinear or unimodal responses, so a canonical correspondence analysis was chosen as a constrained ordination method. Because unimodal methods cannot treat empty cases (in the present study squares with none of the 13 dominant

species present), only 62 out of 78 cases were analysed. Season and year of fieldwork were introduced into the model as covariables to partial out possible confounding effects varying between fieldwork date using partial constrained analysis. The option "down-weight rare species" was not selected (Glavičić et al. 2016). The first canonical correspondence analysis was performed with all 17 environmental variables, and after forward selection 14 variables were incorporated in the final model. In order to avoid inflated significance values, three redundant predictor variables showing high multicollinearity (VIF>10) were removed from the model (temperature, bedrock and no cover) (Table 2). Significance of canonical axes and of the environmental variables was determined using Monte Carlo permutation tests (999 permutations). Significance of canonical axes was tested on the first axis and also on all axes. Significance of the environmental variables is the significance of the conditional (partial) effect of each variable after the forward selection procedure.

The Fisher exact test, ANOSIM and ANOVA were performed in R software version 3.3.3 using the RStudio 1.0.143 integrated development environment (IDE) for R and packages Vegan version 2.4-4., Outliers version 0.14 and Stats version 3.4.1. For the non-metric multi-dimensional scaling, detrended correspondence analysis and canonical correspondence analysis, the Canoco 5 computer package was used (Ter Braak and Šmilauer 2012).

RESULTS

Diversity, abundance and frequency of cryptobenthic and epibenthic fish

The total number of recorded cryptobenthic and epibenthic fish species in the bottom squares was 27 (Table 1). Epibenthic specimens belonged to 13 species and cryptobenthic specimens to 22 species, with eight shared species occurring in both space layers. Among those eight species, *M. macrocephalus* and *Z. zebrus* had predominantly cryptobenthic specimens (98% and 95.1%). The family Gobiidae dominated fish diversity with 14 species, which is more than a half of all recorded species, followed by the family Blenniidae with six species, while the remaining six families were restricted to one or two species (Table 1). Cryptobenthic fish, with 231 individuals collected, highly outnumbered the 31 recorded epibenthic individuals. The average total abundance of cryptobenthic specimens in the bottom squares was 2.92 ± 0.33 individuals/m^2 (mean±SE), with a maximum of 13 individuals/m^2. The average total abundance of epibenthic specimens in the bottom squares was 0.38 ± 0.8 individuals/m^2 (mean±SE), with a maximum of 3 individuals/m^2. The 13 most abundant cryptobenthic species (species with four or more cryptobenthic individuals collected) represented 92.6% of all cryptobenthic individuals and the 5 most abundant species collectively made up 74.0% of all cryptobenthic individuals. The only species with numerous epibenthic individuals recorded was *Scorpaena porcus* Linnaeus, 1758, with all 11

Table 1. – Species occurrence data from the bottom squares of 1 m^2 (n=78), showing recorded species with the number of cryptobenthic (CB) (n=231), epibenthic (EB) (n=31) and total specimens, the percentage of CB specimens and the frequency of species occurrence in the squares. Species in bold letters (species with cryptobenthic occurrence with ≥4 individuals) are included in detrended correspondence analysis and canonical correspondence analysis.

Family	Species (abbrevation)	CB specimens	EB specimens	Total	CB % of total	CB frequency of occurrence	EB frequency of occurrence
Gobiidae	***Corcyrogobius liechtensteini* (Cor lic)**	51	0	51	100.0%	20.5%	0.0%
Gobiidae	***Millerigobius macrocephalus* (Mil mac)**	50	1	51	98.0%	37.2%	1.3%
Gobiidae	***Zebrus zebrus* (Zeb zeb)**	39	2	41	95.1%	32.1%	2.6%
Pomacentridae	***Chromis chromis* (Chr chr)**	17	0	17	100.0%	12.8%	0.0%
Gobiidae	***Chromogobius zebratus* (Chr zeb)**	14	0	14	100.0%	16.7%	0.0%
Scorpaenidae	*Scorpaena porcus* (Sco por)	0	11	11	0.0%	0.0%	12.8%
Tripterygiidae	***Tripterygion melanurum* (Tri mel)**	6	3	9	66.7%	7.7%	3.8%
Gobiidae	***Gobius bucchichi* (Gob buc)**	4	3	7	57.1%	5.1%	2.6%
Gobiidae	***Gobius incognitus* (Gob inc)**	6	1	7	85.7%	7.7%	1.3%
Blenniidae	***Parablennnius zvonimiri* (Par zvo)**	7	0	7	100.0%	9.0%	0.0%
Gobiidae	***Gobius fallax* (Gob fal)**	6	0	6	100.0%	3.8%	0.0%
Blenniidae	***Parablennnius incognitus* (Par inc)**	5	1	6	83.3%	6.4%	1.3%
Gobiesocidae	***Lepadogaster lepadogaster* (Lep lep)**	5	0	5	100.0%	5.1%	0.0%
Gobiidae	***Chromogobius quadrivittatus* (Chr qua)**	4	0	4	100.0%	5.1%	0.0%
Blenniidae	*Parablennnius rouxi* (Par rou)	3	1	4	75.0%	2.6%	1.3%
Gobiidae	*Pomatoschistus bathi* (Pom bat)	0	4	4	0.0%	0.0%	2.6%
Gobiidae	*Odondebuenia balearica* (Odo bal)	3	0	3	100.0%	3.8%	0.0%
Gobiidae	*Didogobius splechtnai* (Did spl)	2	0	2	100.0%	2.6%	0.0%
Lottidae	*Gaidropsarus mediterraneus* (Gai med)	2	0	2	100.0%	2.6%	0.0%
Gobiidae	*Gobius paganellus* (Gob pag)	2	0	2	100.0%	2.6%	0.0%
Blenniidae	*Parablennnius gattorugine* (Par gat)	2	0	2	100.0%	2.6%	0.0%
Tripterygiidae	*Tripterygion tripteronotum* (Tri tri)	1	1	2	50.0%	1.3%	1.3%
Gobiidae	*Gobius cruentatus* (Gob cru)	0	1	1	0.0%	0.0%	1.3%
Gobiidae	*Gobius geniporus* (Gob gen)	0	1	1	0.0%	0.0%	1.3%
Blenniidae	*Microlipophrys adriaticus* (Mic adr)	1	0	1	100.0%	1.3%	0.0%
Blenniidae	*Parablennnius sanguinolentus* (Par san)	0	1	1	0.0%	0.0%	1.3%
Sciaenidae	*Sciaena umbra* (Sci umb)	1	0	1	100.0%	1.3%	0.0%

Table 2. – Environmental variables including all possible variables, with the unrecorded variables in italics letters. The simple (marginal) effect is the amount of variability in the response data that is explained by a constrained ordination model using that variable as the only explanatory variable. The conditional (partial) effect is the additional amount of explained variance in species distribution of the corresponding variable when 14 environmental variables are selected by "blind" forward selection in Canoco 5. The variation in species distribution explained (Explains%) by 14 environmental variables included in the final model by the forward selection procedure are represented with pseudo-F value and P for conditional effect. The categorial variables part of day and number of layers were decomposed in Canoco 5 into daylight, sunset and night and into no layers, one layer and multiple layers, respectively. Environmental variables with a significant conditional effect (p≤0.05) are in bold.

Environmental variable	Simple effects Explains%	Conditional effects Explains%	pseudo-F	P
Time of day: daylight	2.2	1.6	1.1	0.351
Time of day: sunset	1.2	1.6	1.1	0.32
Time of day: night	2.1	1.9	1.3	0.211
Depth	6.5	2.5	1.7	0.091
Temperature	n/a	n/a	n/a	n/a
Inclination (as five estimated classes)	**5.4**	**4.5**	**12.9**	**0.002**
Sand (%)	1.2	0.3	0.2	0.993
Gravel (%)	1.4	1.0	0.7	0.725
Cobbles (%)	**6.3**	**3.6**	**2.3**	**0.008**
Boulders (%)	3.6	1.0	0.7	0.762
Bedrock (%)	n/a	n/a	n/a	n/a
Number of layers: no layers	3.6	2.3	1.6	0.083
Number of layers: one layer	2.8	2.3	1.6	0.115
Number of layers: multiple layers	**6.7**	**6.7**	**4.2**	**0.001**
Phanerogams (%)	n/a	n/a	n/a	n/a
Short tallus algae (%)	1.8	1.1	0.7	0.693
Long tallus algae (%)	n/a	n/a	n/a	n/a
Calcerous algae (%)	3.2	0.5	0.3	0.971
Zoo cover (%)	2.5	1.6	1.1	0.352
No cover (%)	n/a	n/a	n/a	n/a
Number of hidden spaces of entrance size 25-99 cm^2	1.2	2.3	1.6	0.118
Number of hidden spaces of entrance size 100-499 cm^2	3.4	1.5	1.0	0.404
Number of hidden spaces of entrance size 500-1999 cm^2	1.8	1.7	1.2	0.26
Number of hidden spaces of entrance size >2000 cm^2	n/a	n/a	n/a	n/a

individuals recorded on open bottoms, and it is also the only species with just epibenthic specimens recorded. Other species with epibenthic specimens had, in addition to collected cryptobenthic individuals, one to three epibenthic specimens recorded per species. Species of the family Gobiidae contributed to fish abundance with 78.4% of all cryptobenthic individuals sampled and the second most abundant family, Blennidae, with 7.8%. The five most numerous species with cryptobenthic individuals were also the most frequent species in
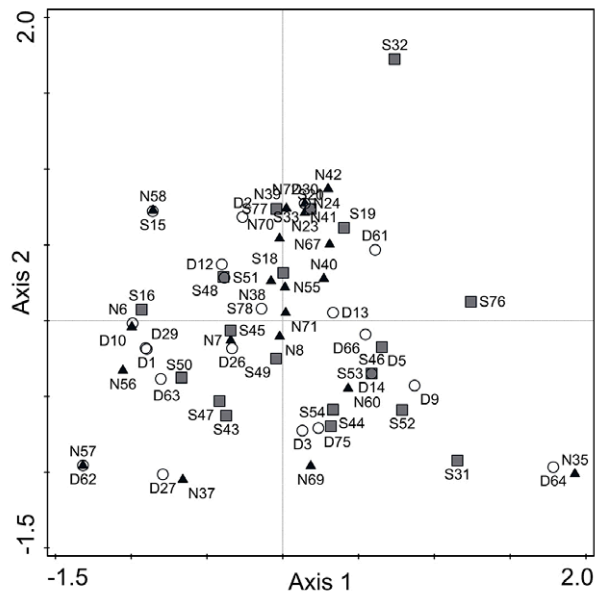
Fig. 1. – Non-metric multi-dimensional scaling ordination plot of individual squares comparing diurnal (hollow circles), sunset (grey quadrats) and nocturnal (black triangles) cryptobenthic fish assemblage species composition.

squares, ranked in presence from about 1/8 to about 2/5 of all squares (Table 1).

Comparison of cryptobenthic and epibenthic fish assemblages

Cryptobenthic and epibenthic fish assemblages showed a significantly different species composition for all data (ANOSIM R=0.2949; p=0.001) and also for each time of day: at daylight (ANOSIM R=0.22; p=0.006), at sunset (ANOSIM R=0.3978; p=0.003) and at night (ANOSIM R=0.3221; p=0.001). Even the qualitative data alone showed the clear difference between the two assemblages, with a limited number of shared or ambivalent species (Table 1). Among species recorded in the cryptobenthic space, 63.6% were exclusively cryptobenthic, and among species recorded in the epibenthic space, 38.5% were exclusively epibenthic.

Diel influence on the cryptobenthic fish assemblage

There was no separation on the non-metric multi-dimensional scaling ordination plot between the diurnal, sunset and nocturnal cryptobenthic fish as-

semblage species composition (stress=0.063) (Fig. 1). The cryptobenthic fish assemblage showed no significantly different species composition between daylight, night and sunset samples in total (ANOSIM R=0.01; p=0.274) and in pairwise comparison of daylight vs. night (ANOSIM R=0.04432; p=0.114), daylight vs. sunset (ANOSIM R=–0.01622; p=0.674) and night vs. sunset (ANOSIM R=0.00715; p=0.334). In addition, R values were close to 0, meaning that there was no separation between levels of the time of the day, so this factor had not just no significance but also no detectable effect at all on cryptobenthic fish assemblage species composition.

Further, one-way ANOVA did not detect differences between different times of day either for species richness of cryptobenthic fish assemblage (d.f.=2, MS=0.3323, F=1.568, p=0.215) or for total fish abundance of the cryptobenthic fish assemblage (d.f.=2, MS=0.7072, F=1.507, p=0.228).

Diel variation of ambivalent species presence in open and hidden spaces

The data for all ambivalent species were combined, and significance was tested on combined frequencies with the Fisher exact test. No significantly different epibenthic and cryptobenthic frequencies of occurrence were found in any data subset between daylight and night, between night and sunset or between daylight and sunset (Table 3), meaning that with the available data it was not possible to identify any significant switch from open to hidden places or vice versa for ambivalent species between times of day. *M. macrocephalus* and *Z. zebrus*, two species with very few epibenthic records, had sufficiently large frequencies at different time of day to be tested separately with the Fisher exact test and also showed no significance difference, i.e. no evidence of utilization of hidden and open spaces at different times of day was established (Table 3).

Important habitat variables structuring the cryptobenthic fish assemblage

The first partial canonical correspondence analysis was performed with 17 environmental variables on 13 species as response variables (Fig. 2A). The Monte Carlo permutation test was statistically significant on the first axis and all other axes (Table 4A). The cumulative percentage of variance in the response

Table 3. – The frequencies of cryptobenthic and epibenthic specimens at the different times of day, summed together for ambivalent species and separately for the predominantly cryptic *Millerigobius microcephalus* and *Zebrus zebrus,* with the results of the Fisher exact test on these frequencies at the different times of day.

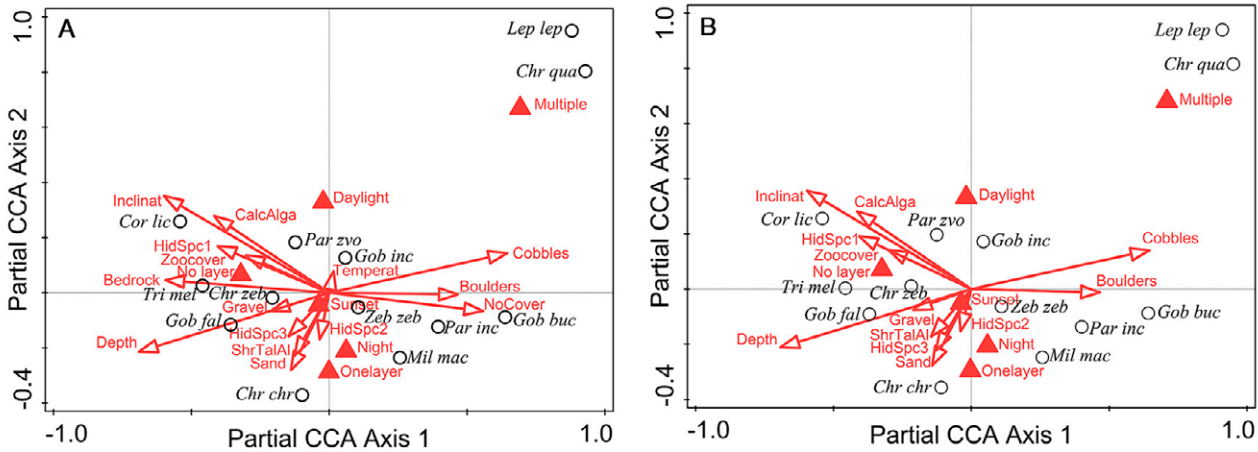| Frequencies	All ambivalent species summed together		*M. macrocephalus*		*Z. zebrus*	
	cryptobenthic	epibenthic	cryptobenthic	epibenthic	cryptobenthic	epibenthic
Daylight	9	6	7	0	12	0
Night	8	3	23	1	8	2
Sunset	8	1	20	0	19	0
Fisher exact test	p-value		p-value		p-value	
Daylight vs. night	0.6828		1		0.1948	
Night vs. sunset	0.5913		1		0.1108	
Daylight vs. sunset	0.1907		1		1	

Fig. 2. – Canonical correspondence analysis species–environment biplot for 17 (A) and 14 (B) predictors (with a focus on response variable distances and biplot scaling) based on analysis of 62 cases with data on abundance of 13 fish species. Summary statistics of the biplot are presented in Table 4. Quantitative environmental variables are represented by red arrows, and each category of factors is indicated by a full red triangle, both with abbreviations in regular font. The abbreviations for explanatory variables: Temperat, temperature; Inclinat, inclination; Onelayer, one layer; Multiple, multiple layers; ShrTalAl, short tallus algae; CalcAlga, calcerous algae; Zoocover, zoo cover; NoCover, no cover; HidSpc1, number of hidden spaces of entrance size 25-99 cm²; HidSpc2, number of hidden spaces of entrance size 100-499 cm²; HidSpc3, number of hidden spaces of entrance size 500-1999 cm². Hollow circles and abbreviations in italics represent fish species. The abbreviations for fish species are explained in Table 1.

data explained by the first four constrained axes was 25.12%, and the first four axes explained 72.83% of variation in the response data explained by explanatory variables, i.e. of the variation of fitted response values (Table 4A). Total variation and partial variation of all eigenvalues are presented in Table 4. The model with 14 variables (after the forward selection procedure excluded redundant variables of temperature, bedrock and no cover) showed slightly lower cumulative explained variation and slightly higher cumulative fitted explained variation than the model with 17 environmental variables (Fig. 2B, Table 4B). Canonical eigenvalues were also slightly lower from the forward selection procedure (Table 4), resulting in explanatory variables accounting for 34.0% of partial variation vs. 34.5% in the model with 17 environmental variables. The conditional effect of daylight, night and sunset in the forward selection procedure was not significant in

explaining species response variables (Table 2). Even simple effects of each time of day were not significant (daylight P=0.229, night P=0.257 and sunset P=0.77). Therefore, the time of day did not reveal a significant correlation with cryptobenthic fish composition and abundance. The forward selection identified three highly significant variables: multiple layers, inclination and cobbles (Table 2), all others being non-significant. After forward selection, the three highly significant variables explained 14.8% of total variation excluding the effects of the covariates, contributing 42.6% to the variation explained by the complete set of explanatory variables. Among highly significant variables, the first axis was positively correlated with inclination and negatively correlated with cobbles, while a further positive correlation was detectable with another quantitative predictor of a large but not significant effect, depth (Fig. 2, Table 2). The projection of the signifi-

Table 4. – Summary statistics of partial canonical correspondence analysis on the distribution of the 13 most common cryptobenthic fishes over 62 sample sites in relation to (A) 17 and (B) 14 environmental variables.

A
Total variation=4.76
All eigenvalues=4.51092

Canonical eigenvalues=1.55566	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.5083	0.2830	0.1879	0.1538
Explained variation (cumulative)	11.27	17.54	21.71	25.12
Pseudo-canonical correlation	0.9045	0.6871	0.7137	0.6175
Explained fitted variation (cumulative)	32.67	50.87	62.95	72.83
Monte Carlo test (P-value, 999 permutations)	0.007	All axes=0.023		

B
Total variation=4.76
All eigenvalues=4.51092

Canonical eigenvalues=1.5333	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.5064	0.2789	0.1856	0.1537
Explained variation (cumulative)	11.23	17.41	21.52	24.93
Pseudo-canonical correlation	0.9018	0.6822	0.7125	0.6156
Explained fitted variation (cumulative)	33.03	51.22	63.32	73.34
Monte Carlo test (p-value, 999 permutations)	The significance of conditional (partial) effect of each variables are reported in Table 2			

cant explanatory factor variable multiple layers onto the arrow lines of cobbles and boulders shows high average value of these quantitative explanatory variables in cases of the multiple layers class (Fig. 2). The most notable small distance between species point and the centroid of the explanatory factor variable predicting high relative species abundance in the class is that of *Lepadogaster lepadogaster* (Bonnaterre, 1788) and *Chromogobius quadrivittatus* (Steindachner, 1863) to multiple layers. The projections of species points of *Corcyrogobius liechtensteini* (Kolombatović, 1891) to inclination and of *Gobius bucchichi* Steindachner, 1870 to boulders show species optima at high values of these quantitative explanatory variables. On the other hand, *L. lepadogaster* and *C. quadrivittatus* species optima are at very small values of depth. The species optima estimated by the calibration variable arrow tool was inclination between steep and very steep to vertical for *C. liechtensteini*, more than 30% of boulders cover for *G. bucchichi* and less than 1 m depth for *L. lepadogaster* and *C. quadrivittatus*.

DISCUSSION

Overall species composition of cryptobenthic and epibenthic fish assemblages differed significantly. This overall differentiation remained stable over the course of the day. In the explicit testing for diel changes in species composition, abundance and richness of the cryptobenthic fish assemblage and for diel changes in habitat choice of ambivalent species, in our study we detected no significant differences. These results suggest that the cryptobenthic fish assemblage has diel stability and that it is mostly composed of permanent inhabitants of hidden spaces with a numerical dominance of miniature gobies. We can hypothesize that, having temporal stability, in the bottom hidden spaces there is a permanent resident cryptobenthic fish community distinct from the epibenthic fish community, i.e. that which is on the bottom surface above them. If species composition in cryptobenthic habitats had drastically changed over different times of day, that would indicate that hidden spaces are mostly daytime shelters inhabited in circadian rhythm mostly by non-strictly cryptobenthic and specialized fishes. This cryptobenthic fish community inhabits various hidden bottom microhabitats and is shaped, according to the results of the present study, mainly by the number of layers present in their respective habitats (multiple layers), bottom inclination, type of layer particles (cobbles) and depth.

Cryptobenthic and epibenthic fish communities in the present study were dominated by gobiid species (14 species) and blennies (Blenniidae, six species), while other families were represented each by only one or two species (Table 1). Cryptobenthic and epibenthic fish communities exhibited a significantly different species compositions across all samples. About two thirds of the species recorded in cryptobenthic position were exclusively cryptobenthic, and more than one third of the species recorded in epibenthic position were exclusively epibenthic. The four most frequent gobiid species, i.e. *C. liechtensteini*, *M. macrocepha-*

lus, *Z. zebrus* and *C. zebratus*, had exclusively or predominantly cryptobenthic specimens, with no evidence for regular or significant habitat switches between cryptobenthic and epibenthic layers (Table 1). Similar to the findings of comparable studies, more than half the specimens sampled in our study belonged to the three most frequent species, all three gobies (Gobiidae) of minute size (Kovačić et al. 2012, Glavičić et al. 2016, Goatley et al. 2016). Among those five most frequent species, *C. liechtensteini*, *Z. zebrus* and *Chromogobius zebratus* were confirmed to be cryptobenthic and abundant, as found by Kovačić et al. (2012) and Glavičić et al. (2016), whereas *M. macrocephalus* was absent or poorly represented in earlier studies due to the deeper depth ranges targeted by Kovačić et al. (2012) and Glavičić et al. (2016). The fourth most frequent species, *C. chromis*, was a damselfish (Pomacentridae), whose adults are known to be hyperbenthic to benthopelagic (Abel 1961). The exclusively cryptic sample of *C. chromis* in the present study confirms previous findings on use of cryptic spaces of this species, which is otherwise present in the water column. The use of cryptic spaces of this species was first found for juvenile fish at night-time (Harmelin 1987), and it was recently also reported in cryptic spaces in daylight (Kovačić et al. 2012, Glavičić et al. 2016). The species is obviously a switcher between hidden spaces and the water column. Since the present method did not sample the water column, the study revealed the exclusively cryptic sample of *C. chromis*. Specimens of all five dominant fish species accounted for approximately 75% of all sampled individuals, whereas the remaining approximately 25% of specimens belonged to 22 species. Their low frequency precluded statistical testing for positive species-specific assignations to hidden or open bottom spaces, with the notable exception of *S. porcus* (Scorpaenidae), whose 11 specimens were exclusively found in epibenthic positions. Cryptobenthic individuals highly outnumbered epibenthic individuals, with a ratio of 7.5, matching results of earlier studies in the Mediterranean Sea (Kovačić et al. 2012: 7.8, Glavičić et al. 2016: 3.6). This further supports the significance of cryptobenthic fishes for the benthos in particular and for the complete ecosystem of infralittoral and circalittoral bottoms if they are rich in cryptic spaces.

Our results highlight the overall importance of diel day/night analyses in fish community studies. To confirm the general temporal stability of the cryptobenthic fish assemblage and complement present day/night analyses, we suggest additional studies on the seasonal changes in cryptobenthic fish assemblages. Future researches should also have alternative approaches to study the cryptobenthic fish community and the trophodynamic functionality of small hidden spaces on sea bottoms. There are virtually not data on the ecological significance of small cryptic spaces on Mediterranean bottoms. The composition, abundance and functioning of sessile and mobile cryptobenthic invertebrates and fishes in these habitats are unknown. Though tropical studies of cryptic spaces are very rare and hard to perform, Scheffers et al. (2003) estimated that the cryptic

space volume could account for 30%-75% of the total coral reef volume; and coelobites, i.e. cryptobenthic organisms living in these spaces, have been calculated to be responsible for phytoplankton removal equivalent to 22% of the gross community metabolism of the entire reef (Richter et al. 2001). To shed light on the ecology of small benthic cryptic spaces on Mediterranean bottoms and the role of cryptobenthic fishes inhabiting them, a comparative stable nitrogen and carbon isotopic study within and across open and hidden bottom spaces in the Adriatic Sea is now being undertaken by a team of researchers, including one of the present authors (M.K.). The stomach content analysis of night and day in situ samples of dominant cryptobenthic fish species is also one of the possible directions of research into the problem of where and how these species are included in the trophic web. Targeted in situ or aquarium observations of these species would further help to understand their autecology and foraging behaviour, and thus help to confirm indirect observations such as those presented here. Our quantitative day/night analyses of cryptobenthic fish community composition patterns is just the first step in research aimed at understanding the processes occurring in the small hidden spaces of Mediterranean littoral bottoms and the role of cryptobenthic fish species in these processes.

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REFERENCES

Abel E.F. 1961. Freiwasserstudien über das Fortpflanzungsverhalten des Mönchfisches *Chromis chromis* Linne, einem Vertreter der Pomacentriden im Mittelmeer. Z. Tierpsychol. 18: 441-449.
https://doi.org/10.1111/j.1439-0310.1961.tb00235.x

Ackerman J.L., Bellwood D.R. 2000. Reef fish assemblages: a re-evaluation using enclosed rotenone stations. Mar. Ecol. Prog. Ser. 206: 227-237.
https://doi.org/10.3354/meps206227

Ahmadia G.N., Pezold F.L., Smith D.J. 2012. Cryptobenthic fish biodiversity and microhabitat use in healthy and degraded coral reefs in SE Sulawesi, Indonesia. Mar. Biodivers. 42: 433-442.
https://doi.org/10.1007/s12526-012-0118-3

Azzurro E., Pais A., Consoli P., et al. 2007. Evaluating day-night changes in shallow Mediterranean rocky reef fish assemblages by visual census. Mar. Biol. 151: 2245-2253.
https://doi.org/10.1007/s00227-007-0661-9

Azzurro E., Aguzzi J., Maynou F., et al. 2013. Diel rhythms in shallow Mediterranean rocky-reef fishes: a chronobiological approach with the help of trained volunteers. J. Mar. Biol. Ass. UK 932: 461-470.
https://doi.org/10.1017/S0025315412001166

Beldade R., Gonçavles E.J. 2007. An interference visual census technique applied to cryptobenthic fish assemblages. Vie Milieu 57: 61-65.

Brandl S.J., Goatley C.H.R., Bellwood D.R., et al. 2018. The hidden half: ecology and evolution of cryptobenthic fishes on coral reefs. Biol. Rev. 93: 1846-1873.
https://doi.org/10.1111/brv.12423

Clarke K.R. 1993. Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18: 117-143.
https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Depczynski M., Bellwood D.R. 2003. The role of cryptobenthic reef fishes in coral reef trophodynamics. Mar. Ecol. Prog. Ser. 256: 183-191.
https://doi.org/10.3354/meps256183

Dulčić J., Fencil M., Matić-Skoko S., et al. 2004. Diel catch variations in a shallow-water fish assemblage at Duće-Glava, eastern Adriatic Croatian coast. J. Mar. Biol. Ass. UK 84: 659-664.
https://doi.org/10.1017/S0025315404009701h

Dulčić J., Matić-Skoko S., Kraljević M., et al. 2005. Seasonality of a fish assemblage in shallow waters of Duće-Glava, eastern middle Adriatic. Cybium 29: 57-63.

Glavičić I., Paliska D., Soldo A., et al. 2016. A quantitative assessment of the cryptobenthic fish assemblage at deep littoral cliffs in the Mediterranean. Sci. Mar. 80: 329-337.
https://doi.org/10.3989/scimar.04307.23A

Goatley C.H.R., González-Cabello A., Bellwood D.R. 2016. Reef-scale partitioning of cryptobenthic fish assemblages across the Great Barrier Reef, Australia. Mar. Ecol. Prog. Ser. 544: 271-280.
https://doi.org/10.3354/meps11614

Goatley C.H.R., Brandl S.J. 2017. Crypthobenthic reef fishes. Curr. Biol. 27: R431-R435.
https://doi.org/10.1016/j.cub.2017.03.051

Harmelin J.-G. 1987. Structure et variabilité de l'ichtyofaune d'une zone rocheuse protégé en Méditerraneé (Pare national de Port-Cros, France). PSZN I: Mar. Ecol. 8: 263-284.
https://doi.org/10.1111/j.1439-0485.1987.tb00188.x

Hobson E.S. 1965. Diurnal-nocturnal activity of some inshore fishes in the Gulf of California. Copeia 1965: 291-302.
https://doi.org/10.2307/1440790

Kovačić M., Patzner R.A., Schliewen U.K. 2012. A first quantitative assessment of the ecology of cryptobenthic fishes in the Mediterranean Sea. Mar. Biol. 159: 2731-2742.
https://doi.org/10.1007/s00227-012-2030-6

McDonald J.H. 2014. Handbook of Biological Statistics. Sparky House Publishing, Baltimore, Maryland, 296 pp.

Prochazka K. 1998. Spatial and trophic partitioning in cryptic fish communities of shallow subtidal reefs in False Bay, South Africa. Environ. Biol. Fish. 51: 201-220.
https://doi.org/10.1023/A:1007407200708

Richter C., Wunsch M., Rasheed M., et al. 2001. Endoscopic exploration of Red Sea coral reefs reveals dense populations of cavity-dwelling sponges. Nature 413: 725-730.
https://doi.org/10.1038/35099547

Scheffers S.R., de Goeij J., van Duyl F.C., et al. 2003. The cave-profiler: a simple tool to describe the 3-D structure of inaccessible coral reef cavities. Coral Reefs 22: 49-53.

Smith-Vaniz W.F., Jelks H.L., Rocha L.A. 2006. Relevance of cryptic fishes in the biodiversity assessments: A case study at Buck Island Reef National Monument, St. Croix. Bull. Mar. Sci. 79: 17-48.

Šmilauer P., Lepš J. 2014. Multivariate analysis of ecological data using CANOCO 5. Cambridge Univ. Press, Cambridge, 362 pp.
https://doi.org/10.1017/CBO9781139627061

Ter Braak C.J.F., Šmilauer P. 2012. Canoco reference manual and user's guide: software for ordination, version 5.0. Microcomputer Power, Ithaca, USA, 496 pp.

Thiriet P.D., Di Franco A., Cheminéeé A., et al. 2016. Abundance and Diversity of Crypto- and Necto-Benthic Coastal Fish Are Higher in Marine Forests than in Structurally Less Complex Macroalgal Assemblages. PLoS ONE 11: e0164121.
https://doi.org/10.1371/journal.pone.0164121

Tornabene L., Ahmadia G.N., Berumen M.L., et al. 2013. Evolution of microhabitat association and morphology in a diverse group of cryptobenthic coral reef fishes (Teleostei: Gobiidae: *Eviota*). Mol. Phylogenet. Evol. 66: 391-400.
https://doi.org/10.1016/j.ympev.2012.10.014

Underwood A.J. 1997. Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge.
https://doi.org/10.1017/CBO9780511806407

Willis T.J. 2001. Visual census methods underestimate density and diversity of cryptic reef fishes. J. Fish Biol. 59: 1408-1411.
https://doi.org/10.1111/j.1095-8649.2001.tb00202.x

Willis T.J., Anderson M.J. 2003. Structure of cryptic reef fish assemblages: relationships with habitat characteristics and predator density. Mar. Ecol. Prog. Ser. 257: 209-221.
https://doi.org/10.3354/meps257209