

# Increasing heterogeneity of sensitive assemblages as a consequence of human impact in submarine caves

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**Abstract** Submarine caves are considered as a top priority for conservation, but the effects of common pressures are poorly known for these habitats. Here, we examined the effect of recreational human visitation on a selection of submarine caves in a Mediterranean Marine Protected Area (40°35'40"N; 8°11'39"E) where diving activities are regulated. Sampling was conducted in visited and not visited caves to assess whether diving activities have a significant effect on cave habitats, what are the components of biodiversity most affected by this disturbance, and its potential effects on spatial heterogeneity of benthic assemblages. Results clearly showed that human visitation could significantly affect spatial patterns of benthic assemblages. Organisms with erect growth forms were significantly more abundant and homogeneously distributed where diving activities are forbidden. An increase in the small-scale heterogeneity of assemblages and a decrease in their three-dimensional structure could be the ultimate consequences of human visitation. The interaction between specific stressors and the patterns of distribution of species and assemblages can drive their spatial heterogeneity also in unique habitats like marine caves, representing an early warning for the development of appropriate management measures.

## Introduction

Understanding how human perturbations combine with natural processes in determining biodiversity patterns is an important challenge in ecological research. Recent work about these processes on marine habitats provided important insights into how species and assemblages are structured and how anthropogenic impacts might be best managed (e.g., Castilla 1999, 2000; Murray et al. 2006). The information is, however, still confined to few habitat types (e.g., rocky intertidal) while others, being less accessible, are still poorly investigated.

Submarine caves represent a paradigmatic example. They are widespread in many tropical and Mediterranean areas (see Ford and Williams 2007) and are featured by unique patterns of compositional diversity (Riedl 1966; Ott and Svoboda 1976; Harmelin 1997). Significant differences, in terms of both benthic invertebrates and fish species richness and assemblage structure, can be observed between caves just tens of meters from each other (Bussotti et al. 2006). Variation in larval and food supply, differences in species colonization on a scale of a few meters, and post-settlement events have been invoked as the main biotic processes driving this variability (Airoldi and Cinelli 1996; Benedetti-Cecchi et al. 1997; Chevaldonné and Lejeune 2003; Denitto et al. 2007). Cave assemblages can also deeply change from the opening to the interior (Bussotti et al. 2006), so that they have been frequently used as model systems to assess the effects of environmental gradients on patterns of distribution of sessile organisms (e.g., Ott and Svoboda 1976; Harmelin 1997; Marti et al. 2004, 2005).

Marine caves are considered as poorly resilient ecosystems (Vacelet et al. 1994), and understanding their potential vulnerability to and recovery from major disturbances is

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mandatory for their management and conservation (Chevaldonné and Lejeune 2003; Parravicini et al. 2010). Marine caves are priority habitats, included in the Habitats Directive (European Union, Council Directive 92/43/EEC). Furthermore, the 56% of the Marine Protected Areas (MPAs) in the Mediterranean Sea includes this habitat (Abdulla et al. 2008), with a combination of protection initiatives. Considering the variety of pressures commonly impinging on coastal systems (Halpern et al. 2007), the information available on the effects of human disturbance is extremely limited for marine caves (Di Franco et al. 2010; Parravicini et al. 2010).

The establishment of MPAs around the world provided opportunities for a rapid growth of “marine-based” tourism (Davis and Tisdell 1995), mainly derived from diving-related activities (e.g., Walls 1998; Badalamenti et al. 2000; Lloret et al. 2008). Ecotourism, as a consequence, has been ranked as a threat to hard- and soft-substratum ecosystems (Halpern et al. 2007). Tourism revenue, measured in terms of dive trips, can strongly increase in MPAs (Worm et al. 2006; Merino et al. 2009). The increase in diving visitation, if not adequately regulated, leads to detrimental effects for benthic assemblages (Milazzo et al. 2002), conflicting with the conservation objectives of MPAs (Hawkins et al. 2005) and thus impairing management initiatives to coupling protection of biodiversity and local economic growth (Davis and Tisdell 1996; Harriott et al. 1997; Walters and Samways 2001).

Although pressures of recreational scuba diving are continuously increasing, a clear management strategy specifically dealing with this potential impact in marine caves under various protection regimes is lacking. Recreational scuba diving includes proximate impacts, such as breaking and abrading vulnerable organisms, as well as indirect impacts such as resuspension of sediment and accumulation of exhaled air bubbles at the ceiling (Milazzo et al. 2002; Lloret et al. 2006). Diver impacts can be cumulative, not only affecting the cover of algae and invertebrates but also community structure and species diversity and distribution. A shift from three-dimensional to flattened habitats is often the outcome of unregulated diving visitation (Lloret et al. 2006), with serious consequences on a critical ecological group, such as organisms with erect growth forms involved in trophic dynamics and calcification processes.

Biotic homogenization (Olden and Rooney 2006) is generally considered to occur at broad scales or in response to high-intensity impacts. More localized anthropogenic disturbance like human frequentation can determine changes both in single response variables (e.g., total number of species or individuals, diversity indices, biomass, or abundance of a single species) (e.g., Sala et al. 1996; Garrabou et al. 1998) and in the multivariate

structure of marine assemblages (see Milazzo et al. 2002 for a review). However, anthropogenic disturbance can also affect local heterogeneity, and increasing variability in pattern of distribution of benthic assemblages are considered to be a sensitive response, occurring before gross changes in mean abundance (Hewitt and Thrush 2009).

Here, we focus on a selection of submarine caves from a Mediterranean MPA where visited and not visited caves coexist to assess whether diving activities have a significant effect on cave habitats, what are the components of biodiversity most affected by visitation, and its potential effects on spatial heterogeneity of cave assemblages.

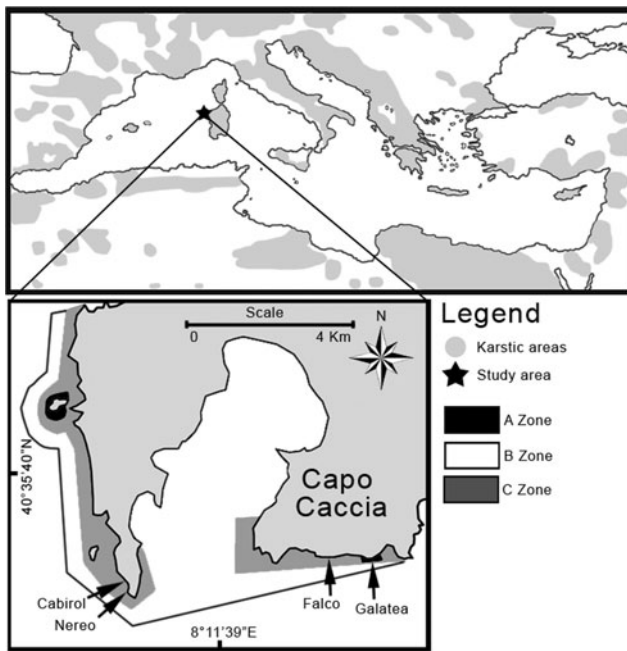
## Materials and methods

### Study site

The study was conducted within the MPA of Capo Caccia-Isola Piana (40°35'40"N; 8°11'39"E), NW coast of Sardinia, Italy (Fig. 1). The MPA has been established in 2002 and covers an area of about 26 km<sup>2</sup>. The whole area is mostly characterized by rocky reefs (mostly calcarenitic substratum), conducive to the formation of cavities on the cliffs (Suric et al. 2010). The MPA has an adequate enforcement and the good relationships between the MPA manager and the local stakeholders insure gathering of realistic information about local human pressures deriving from touristic frequentation on this habitat.

The MPA coastline is characterized by the presence of about 30 caves. A preliminary survey was carried out in order to find comparable caves in terms of morphology (i.e., blind cave tunnel shaped with horizontal development) and depth of entrance ( $\approx 10$  m), exposed and not exposed to human visitation. Four caves were chosen for a formal comparison (Fig. 1): three caves, namely “Grotta del Falco” (about 155 m long), “Grotta di Nereo” ( $\approx 400$  m long), and “Grotta del Cabirol” ( $\approx 30$  m long), outside the no-take zone (A zone), represent traditionally elected sites for diving activities, and one cave not exposed to human visitation (because of formal restrictions), namely “Grotta Galatea” ( $\approx 75$  m long). Information about the levels of visitation for each cave was obtained on the basis of authorizations released by the Managing Direction of the MPA. In this area, recreational scuba diving occurs from May to September. The number of divers per month visiting the three unprotected caves (Falco, Nereo, Cabirol) ranges from 30 to 180. Local diving centers always accompany divers with about 6–10 divers at once.

Sampling activities were conducted in September 2009, immediately after the peak season for tourism-related diving. Independently from the longitudinal development



**Fig. 1** Map of the study area. Marine Protected Area of Capo Caccia: no-take, no-entry areas are given in *black*, the buffer zone of the MPA in *white*, whereas in *gray* the regulated area. Galatea = protected cave; Falco, Cabirol, Nereo = visited caves. The map shows also major carbonate rocks outcrops providing an estimate of karst regions of the Mediterranean area and related caves formation (modified from Ford and Williams 2007)

of each caves, it was decided to sample the first 20 m from the entrance, representing the more attractive and accessible sectors also to inexperienced divers and thus particularly exposed to their putative impact (Di Franco et al. 2009), possibly also caused to buoyancy difficulties. In all caves, this portion was featured by coralligenous and semi-dark cave assemblages.

#### Sampling design

Sampling was undertaken on the rocky walls of the caves by non-destructive photographic methods, using a diving suited Canon EOS 400D digital camera and two electronic strobes. Three sectors (0, 10, and 20 m from the entrance) were sampled in each cave. Three random areas of 3 m<sup>2</sup> each were identified in each sector, and 8 random 16 × 23 cm photo-quadrats were taken in each area (Roberts et al. 1994) for a total of 288 photographic samples. Each sampling unit was analyzed under magnification by superimposing a grid of 24 equally sized squares. Percentage cover values of sessile algae and invertebrates were estimated visually by giving a score from 0 (absence) to 4 (totally covered) to each taxon present on each sub-quadrat and adding up these values over the 24 sub-quadrates (Dethier et al. 1993). Destructive samples were also collected and specialists assisted in the taxonomic

identification of organisms. Organisms that were not easily identifiable at species level were collapsed into higher taxonomic groups or into morphological groups (Steneck and Dethier 1994). Motile animals were not considered in the analysis.

#### Statistical analyses

The analyses were run under the hypothesis that the disturbance by divers could drive changes at assemblage level affecting both cover and patterns of distribution of a suite of species particularly vulnerable to this specific impact. A distance-based permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) was thus performed to test for the effect of visitation on cave assemblages as a whole. The experimental design consisted of three factors: cave (Ca, with one protected and three visited caves), sector (Se, 3 levels, fixed and crossed to Ca), and area (Ar, 3 levels, random and nested within the interaction of the other factors), with  $n = 8$  replicates per combination of factors. Because of the presence of a single protected (Pr) and three visited caves (Vi), the design was asymmetrical. Therefore, for the analyses, the cave term was partitioned into two portions: the contrast of protected versus visited caves (Pr vs. Vi) and the variability between visited caves (Vi). Also the cave × sector (Ca × Se) term was divided into Pr versus Vi × Se and a Vi × Se interaction terms. The same approach was used for the term area (Ca × Se). Also, the residual variation was divided into the variability for observations within Pr (Res Pr) and variability for observations within Vi (Res Vi). Appropriate denominators for  $F$  ratios were identified from expected mean squares, and tests were constructed following the logic of asymmetrical designs (see Terlizzi et al. 2005 for details). The analyses were based on Bray-Curtis dissimilarities calculated on untransformed data, and each term was tested using 4,999 random permutations (Anderson et al. 2008). Multivariate patterns of assemblage variation among caves were visualized through a non-metric multidimensional scaling (nMDS) ordination of Ca × Se centroids. A similarity percentage analysis (SIMPER, Clarke 1993) was employed to identify taxa most contributing to patterns of assemblages' variation between Pr and Vi. Only variables whose contribution exceeded an arbitrarily chosen threshold value of % dissimilarity  $\geq 2.5\%$  were selected.

K-dominance curves (Lambshead et al. 1983) were used to compare diversity among the four caves by displaying the cumulative proportion abundance against the log species rank.

Since the disturbance caused by diving visitation is supposed to act non-randomly, an asymmetrical analysis of variance (ANOVA) following the same design of

**Table 1** Inventory of taxa recorded inside the four caves from photographic samples

Rhodophyceae	<i>Ircinia retidermata</i>	<i>Alcyonium coralloides</i>
<i>Amphiroa rigida</i>	<i>Ircinia variabilis</i>	<i>Cornularia cornucopiae</i>
<i>Corallina elongata</i>	<i>Chondrosia reniformis</i>	<i>Parazoanthus axinellae</i>
<i>Corallina officinalis</i>	<i>Erylus</i> sp.	
<i>Halimeda tuna</i>	<i>Corticium candelabrum</i>	
<i>Tricleocarpa fragilis</i>	<u><i>Petrosia</i> spp.</u>	Polychaeta
Dumontiaceae	Clionidae	Calcareous tube worms
<i>Laurencia</i> sp.	<i>Cliona rhodensis</i>	
<u><i>Lithophyllum stictaeforme</i></u>	<i>Cliona schmidtii</i>	Gastropoda
<i>Peyssonnelia</i> sp.	<i>Cliona viridis</i>	<i>Vermetus</i> sp.
<i>Peyssonnelia crispata</i>	<i>Haliclona (Reniera) mucosa</i>	
	<i>Petrobiona massiliana</i>	Bivalvia
	<i>Clathrina clathrus</i>	<i>Spondylus</i> sp.
Phaeophyceae	<i>Clathrina contorta</i>	<i>Gastrochaena dubia</i>
<i>Dictyota dichotoma</i>	<i>Crambe crambe</i>	<i>Lithophaga lithophaga</i>
	<i>Clathrina rubra</i>	
	<i>Haliclona fulva</i>	
Chlorophyceae	<i>Hemimyscale columella</i>	Cirripedia
<i>Codium effusum</i>	<i>Dendroxea lenis</i>	Balanidae
<i>Palmophyllum crassum</i>	<i>Phorbas</i> spp.	
<i>Flabellia petiolata</i>	<i>Phorbas tenacior</i>	Bryozoa
<i>Valonia</i> spp.	<i>Oscarella</i> spp.	Encrusting bryozoans
	Spirastrellidae	Thin ramified bryozoans
Algal groups	<i>Spirastrella cunctatrix</i>	<i>Patinella radiata</i>
Encrusting calcareous rhodophytes	<i>Diplastrella bistellata</i>	<i>Chlydonia pyriformis</i>
Green filamentous algae	<i>Spongia virgultosa</i>	<u><i>Idmidronea triforis</i></u>
Dark filamentous algae	<i>Terpios fugax</i>	<u><i>Hornera frondiculata</i></u>
Thin tubular or sheet-like	Yellow encrusting sponges	<u><i>Myriapora truncata</i></u>
	Porifera sp.1	<u><i>Reteporella grimaldii</i></u>
	Porifera sp.2	<u><i>Adeonella calveti</i></u>
	Porifera sp.3	<u><i>Smittina cervicornis</i></u>
Foraminifera	Porifera sp.4	<u><i>Carbasea</i> sp.</u>
<i>Miniacina miniacina</i>	Hydrozoa	Brachiopoda
Porifera	Hydroids	<i>Margelia truncata</i>
<u><i>Acanthella acuta</i></u>	<i>Novocrania anomala</i>	
<i>Dictyonella</i> sp.		
<u><i>Cymbaxinella damicornis</i></u>		
<u><i>Pleraplysilla spinifera</i></u>	Anthozoa	
<i>Haliclona mediterranea</i>	Anthozoans	Ascidacea
<i>Dysidea fragilis</i>	<i>Caryophyllia</i> spp.	<i>Ascidia</i> sp.
<i>Dysidea incrustans</i>	<i>Leptopsammia pruvoti</i>	<i>Ciona</i> sp.
<u><i>Agelas oroides</i></u>	<i>Madracis pharensis</i>	<i>Halocynthia papillosa</i>
<i>Fasciospongia cavernosa</i>	<i>Polycyathus muelleriae</i>	<i>Botryllus</i> sp.
<i>Spongia lamella</i>	<u><i>Corallium rubrum</i></u>	Didemnidae
Dictyoceratida	<i>Epizoanthus</i> sp.	<i>Diplosoma</i> sp.

Underlined taxa are considered sensitive to mechanic disturbance (i.e., direct contact) of scuba divers (according to Lloret et al. 2006)

multivariate analysis was run on the total cover of selected taxa (Table 1) that, for their specific morphological traits (e.g., calcareous erect forms), were more likely to be impacted by the mechanical disturbance (i.e., direct contact) resulting from diving activities (Lloret et al. 2006). We focused on these taxa since the putative effects of

additional indirect impacts related to this source of disturbance (i.e., sediment resuspension and accumulation of exhaled air bubbles at the ceiling) on other sessile organisms are idiosyncratic (e.g., Di Franco et al. 2010) because strictly related to cave characteristics (e.g., sediment grain size, cave morphology, inclination of substratum). It is also

expected that mechanical disturbance as a consequence of diving visitation may lead to increasing small-scale patchiness of the sensitive components of assemblages, whereas non-sensitive taxa would be virtually unaffected. To test this hypothesis, a permutational analysis of multivariate dispersion (PERMDISP, Anderson et al. 2006) on the basis of Bray-Curtis dissimilarity was carried out for the factor Pr versus Vi, separately for each sector, both on the same subset of taxa considered in the ANOVA (i.e., sensitive taxa, see Table 1) and on taxa that are considered non-sensitive.

## Results

### Structure of assemblages

Sample analysis allowed the identification of 99 taxa belonging to 10 phyla (Table 1). Most taxa were identified at species (64%) and genus (15%) level. The remaining taxa were aggregated into higher taxonomic and/or morphological groups (Steneck and Dethier 1994). Eight morphological groups were identified: encrusting calcareous rhodophytes (e.g., *Lithophyllum incrustans*, *Lithothamnion sonderi*), yellow encrusting sponges (e.g., *Halisarca* sp., *Crella* sp., and *Merlia* sp.), calcareous tube worms (mainly Serpulidae), encrusting bryozoans (mainly Celleporidae), thin erect bryozoans (e.g., *Bugula* spp., *Scrupocellaria* sp.), turf-forming algae subdivided into green filamentous algae (mainly

Cladophorales), dark filamentous algae (mainly Ceramiales), and thin tubular or sheet-like algae (e.g., *Rhodymenia pseudopalmata* and *Nithophyllum* spp.).

PERMANOVA showed a significant variability at the scale of area (Table 2) for both fully protected (Pseudo- $F_{(6,63)} = 4.55$ ,  $P[\text{Perm}] < 0.001$ ) and visited caves (Pseudo- $F_{(18,189)} = 3.73$ ,  $P[\text{Perm}] < 0.001$ ), confirming the high small-scale variability of assemblages inhabiting this habitat. The analysis also showed significant differences in Pr versus Vi (Table 2) caves (Pseudo- $F_{(1,2)} = 3.58$ ,  $P[\text{Perm}] = 0.0176$ ), indicating a significant effect of human frequentation in modifying the structure of cave assemblages. Patterns of differences between Pr versus Vi, and their consistency across sectors, were clearly depicted in the nMDS of Ca  $\times$  Se centroids (Fig. 2a). A potential homogenization determined by the impact of divers was suggested by the lower variance component observed at small scale among residuals at the visited caves (Table 2). Significant differences were also observed among k-dominance curves (ANOSIM, Global  $R = 0.426$ ,  $P = 0.001$ ), with the distribution of the number of individuals among species at visited caves markedly differing from the protected one, where assemblages were characterized by higher diversity and evenness. The shape of the curves at visited caves was comparable, indicating similar patterns of distribution of individuals among species (Fig. 2b).

SIMPER analysis showed that individual taxa vulnerable to the damage of direct contact of divers, such as erect

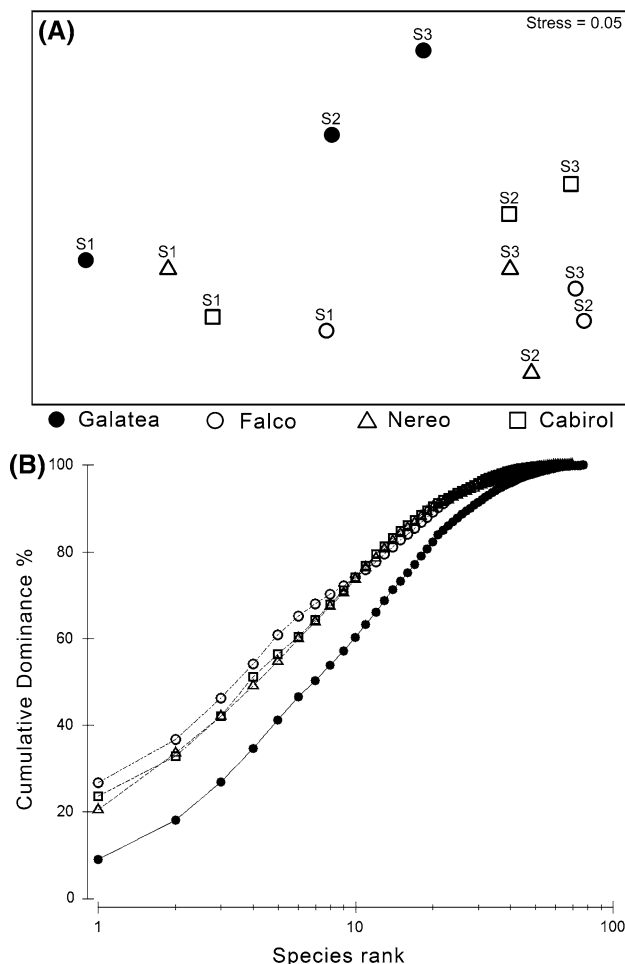
**Table 2** Results of PERMANOVA investigating differences between assemblages in protected versus visited caves. Analysis based on Bray-Curtis dissimilarities (untransformed data) conducted on the whole dataset (99 taxa  $\times$  288 samples)

Source of variation	<i>df</i>	SS	MS	Pseudo- <i>F</i>	<i>P</i> [Perm]	MS <sub>DEN</sub>
Ca	3	123,540	41,180	10.37	0.0002	Ar(Ca $\times$ Se)
Vi	2	44,266	22,133	6.57	0.0002	Ar(Vi $\times$ Se)
Pr versus Vi	1	79,274	79,274	3.58	<b>0.0176</b>	Vi
Se	2	192,800	96,402	5.90	0.0004	Ca $\times$ Se
Ca $\times$ Se	6	98,079	16,346	4.12	0.0002	Ar(Ca $\times$ Se)
Vi $\times$ Se	4	57,155	14,289	4.24	0.0002	Ar(Vi $\times$ Se)
Pr versus Vi $\times$ Se	2	40,924	20,462	1.43	0.2138	Vi $\times$ Se
Ar(Ca $\times$ Se)	24	95,324	3,972	3.99	0.0002	Residuals
Ar(Vi $\times$ Se)	18	60,681	3,371	3.73	0.0002	Residuals Vi
Ar(Pr versus Vi $\times$ Se)	6	34,643	5,774	4.55	0.0002	Residuals Pr
Residuals	252	250,690	995			
Residuals Vi	189	170,710	903			
Residuals Pr	63	79,980	1,270			
Total	287	760,430				

Each test was performed using 4,999 permutations of appropriate units

*P* values (*P*[Perm]) given in italics and bold were obtained using 4,999 Monte Carlo samples from the asymptotic distribution. The term used for the denominator mean square (MS) in each case is given in column MS<sub>DEN</sub>. Terms already involved in significant higher-order interactions were not analyzed. Ca Cave, Vi visited, Pr protected, Se sector, Ar area





**Fig. 2** **a** Non-metric multidimensional scaling ordinations (nMDS) of caves  $\times$  sector centroids based on Bray-Curtis dissimilarity. **b**. K-dominance curves for species abundances at protected and visited caves (x-axis logged). Protected cave = black; visited caves: empty symbols. (S1 = 0 m, S2 = 10 m, S3 = 20 m)

**Table 3** Summary of SIMPER analysis showing taxa most contributing to dissimilarities between protected and visited cave assemblages

Species	Pr Average abundance	Vi Average abundance	Relative contr. (%)	Cumulative contr. (%)
Encrusting bryozoans	2.24	19.33	13.45	13.45
<i>Parazoanthus axinellae</i>	7.51	6.63	6.80	20.25
<i>Dendroxea lenis</i>	7.40	6.63	6.62	26.87
Encrusting calcareous rhodophytes	7.53	4.02	6.56	33.43
<i>Lithophyllum stictaeforme</i>	6.36	1.41	5.15	38.58
<i>Leptopsammia pruvoti</i>	0.12	6.58	5.01	43.59
<i>Idmidronea triforis</i>	5.46	1.25	4.27	47.86
<i>Reteporella grimaldii</i>	4.47	1.61	3.52	51.38
Spirastrellidae	3.01	1.68	2.94	54.32
Calcareous tube worms	2.07	4.82	2.86	57.17
Thin ramified bryozoans	3.13	1.03	2.77	59.94
<i>Peyssonnelia crispata</i>	1.57	2.5	2.56	62.50
<i>Miniacina miniacea</i>	1.40	3.60	2.55	65.05

Pr Protected caves, Vi visited caves

calcified bryozoans (i.e., *Reteporella grimaldii*, *Idmidronea triforis*) and encrusting algal species characterized by calcified thallus (i.e., *Lithophyllum stictaeforme*), strongly contributed to characterize the assemblage of the protected cave (Table 3). The structure of assemblages within visited caves was mainly characterized by encrusting organisms, mainly bryozoans. ANOVA on the total coverage of these vulnerable species showed significant differences between Pr versus Vi ( $F_{(1,2)} = 19.15$ ,  $P = 0.0485$ ), with higher values in the protected cave than in the visited ones (Table 4 and Fig. 3a).

Results of PERMDISP provided further evidence of this effect. Tests on multivariate dispersion showed significant differences between Pr versus Vi in each sector ( $P < 0.01$ ), indicating that the vulnerable component of benthic assemblages had a higher spatial heterogeneity in the visited caves than in the protected one (Fig. 3b). In contrast, results of PERMDISP on non-vulnerable component of assemblages showed no significant differences in small-scale heterogeneity between protected and visited caves (Fig. 3c). The inspection of graph in Fig. 3c shows a general decrease in spatial heterogeneity along the exterior-interior axis (i.e., from 0 to 20 m inside the caves) for this component of assemblages.

## Discussion

Our results suggest that diving visitation affects benthic assemblages, causing significant changes in biodiversity patterns between visited versus protected caves, altering the structure of assemblages and evenness of benthic taxa. We are aware that the unique features of this habitat are generally conducive to conceptual and methodological

**Table 4** Results of ANOVA investigating differences between the total coverage of vulnerable species in protected versus visited caves

Source of variation	<i>df</i>	SS	MS	<i>F</i>	<i>P</i>	MS <sub>DEN</sub>
Ca	3	11,361	3,787	16.40	0.0000	Ar(Ca × Se)
Vi	2	1,065	533	6.56	0.0073	Ar(Vi × Se)
Pr versus Vi	1	10,196	10,195	19.15	0.0485	Vi
Se	2	156	78	0.52	0.6166	Ca × Se
Ca × Se	6	891	149	0.64	0.6948	Ar(Ca × Se)
Vi × Se	4	610	153	1.88	0.1582	Ar(Vi × Se)
Pr versus Vi × Se	2	281	141	0.92	0.4682	Vi × Se
Ar(Ca × Se)	24	5,543	231	4.09	0.0000	Residuals
Ar(Vi × Se)	18	1,462	81	2.00	0.0114	Residuals Vi
Ar(Pr versus Vi × Se)	6	4,081	680	6.52	0.0000	Residuals Pr
Residuals	252	14,232	56			
Residuals Vi	189	7,664	41			
Residuals Pr	63	6,569	104			
Total	287	32,183				

The term used for the denominator mean square (MS) in each case is given in column MS<sub>DEN</sub>. *Ca* Cave, *Vi* visited, *Pr* protected, *Se* sector, *Ar* area

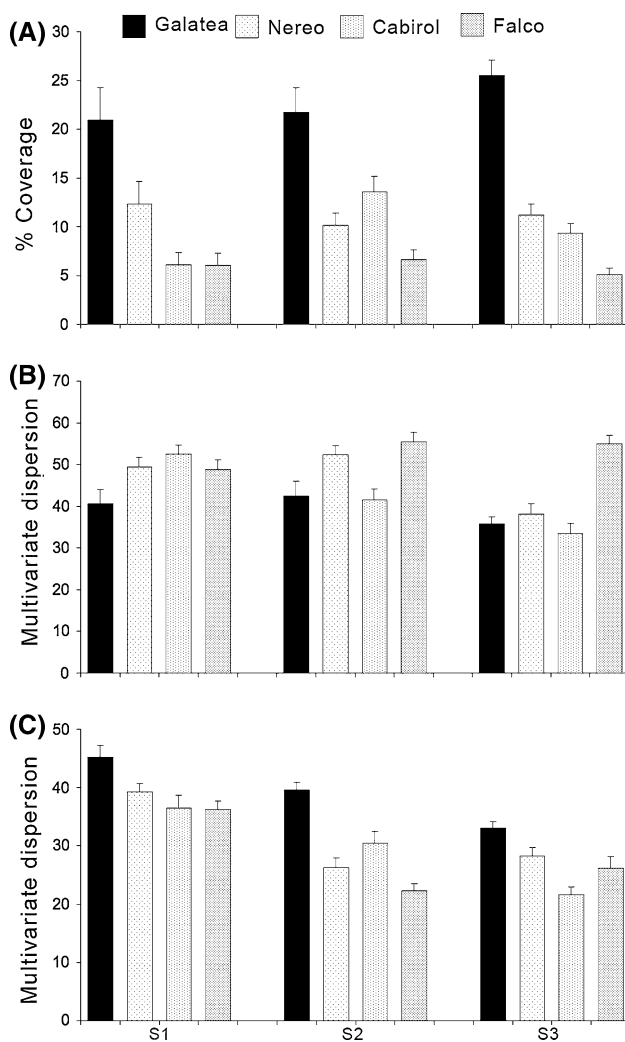
constrains limiting the possibility of formal comparisons following the logic of asymmetrical designs (Glasby 1997). Taking into account only one cave in the non-visited condition, as in our case, could impair the potential to assess whether or not differences within visited and non-visited caves are greater, similar, or lower than between visited and non-visited caves and thus to ascertain whether the observed patterns of variation are imputable to diving visitation or to natural variability. Given that, our results provide some interesting insights into the effects of human disturbance on cave assemblages.

We found that diving visitation strongly affected selectively the small-scale pattern of heterogeneity of the component of assemblages sensitive to mechanical damage. This effect of diving visitation went over and beyond the high natural variability among caves. The impact of visitation is non-random, since some taxa (such as some bioconstructors, Ballesteros 2006) are highly susceptible to damage by direct contact of divers (Sala et al. 1996; Garrabou et al. 1998; Lloret et al. 2006) due to their morphological and structural traits (e.g., vertical growth form, rigid body structure). These taxa (e.g., *Lithophyllum stictaeforme*, *Reteporella grimaldii*, *Idmidronea triforis*) are significantly more abundant and homogeneously distributed in the fully protected cave than in the visited ones. In contrast, as expected, non-sensitive taxa showed no differences in small-scale patchiness between protected and visited caves. These taxa, especially encrusting growth forms, less exposed to contact damage (e.g., encrusting bryozoans), characterized visited caves suggesting that the impact from diving visitation likely determines a shift from

a three-dimensional to a flattened habitat, with relevant but underestimated consequences on cave habitats.

Low level of disturbance may represent an important driver of biodiversity (Sousa 2001). Asynchronous dynamics of individual patches are expected to produce heterogeneity at low levels of disturbance, with emerging mosaics of patches of different successional stages, increasing overall species richness (Hewitt et al. 2010). However, the potential for relative shifts in the number of species (average, total, and heterogeneity) at small spatial scales can be the symptom of ongoing biodiversity loss as a result of low levels of human disturbance (Fraschetti et al. 2001). For instance, when acting non-randomly on the different components of assemblages, even low levels of disturbance could negatively affect biodiversity by causing the decline or disappearance of selected taxa. In this framework, our results suggest that the increasing small-scale heterogeneity of sensitive component of cave assemblages, with the dominance of less vulnerable, more robust species, can be the early signal of an ecological shift associated with low level of disturbance (Hewitt et al. 2010), as a consequence of human frequentation.

Scuba diving represents a seasonal small-scale threat. It has adverse consequences on benthic assemblages, because divers can easily damage marine organisms through physical contact with their hands, body, equipment, and fins (Rouphael and Inglis 1997; Tratalos and Austin 2001; Zakai and Chadwick-Furman 2002; Uyarra and Cote 2007). Di Franco et al. (2009) clearly showed that the rates of contact are particularly high in caves and encrusted walls. According to their observations, based on the



**Fig. 3** **a** Average percent coverage ( $\pm$  SE,  $N = 24$ ) of vulnerable species and **b**, their multivariate dispersion (PERMDISP) within protected and unprotected caves split for the different sectors (S1 = 0 m, S2 = 10 m, S3 = 20 m). **c**, Multivariate dispersion of non-vulnerable component of assemblages. Black bars = not visited, White bars patterned = visited

conservative assumption that during August, in the visited caves, 6 divers/day have a dive lasting 30 min each, 4,486 contacts with the substratum in only 1 month can occur, causing permanent injuries. However, the magnitude of disturbance can be poorly correlated to the number of divers that visit a given site (Barker and Roberts 2004), because it is strongly influenced by both the experience and behavior of the divers (Di Franco et al. 2009; Luna et al. 2009) and the physical and biological features of the site (Garrahou et al. 1998; Tratalos and Austin 2001).

Our findings support the management and conservation of cave assemblages in three ways. First, at local scale, these results can help refining threshold levels for frequenting caves and suggest that different visitation levels might be set depending on the characteristics of natural

assemblages. Second, *ad hoc* briefing should indicate to divers the taxa (e.g., erect growth forms) that are most vulnerable to the disturbance they might cause. Third, monitoring programs for adaptive management should focus on different structural properties of assemblages, including spatial heterogeneity, which could highlight subtle effects of human disturbance before the occurring of more drastic changes such as biodiversity losses or even regime shifts.

MPAs are commonly considered as being effective to ensure ecological sustainability, while also keeping human use options open (Davis and Tisdell 1995). However, partially protected areas may confer some benefits over open access areas, but no-take reserves generally show greater benefits and yield significantly higher densities of organisms within their boundaries relative to partially protected sites nearby (Lester and Halpern 2008). The opening of MPAs at multiple uses represents an example of effective management of marine resources, through both the protection of marine biodiversity and, contemporarily, the reduction of conflicts among users (Sorice et al. 2007; Parsons and Thur 2008). The assessment of human impacts deriving from allowed activities inside MPAs, such as scuba diving, represents a prerequisite for setting *ad hoc* regulation of MPA's use destination (Garrahou et al. 1998). Our results show that management practice based on a precautionary recreational threshold level should be carefully considered because subtle effects of diving could occur at low levels of visitation as those documented in this study (i.e., up to 180 divers per month).

In general, studies aimed at quantifying carrying capacities for different human uses of marine ecosystems, while accounting for co-occurring disturbances and natural variation in environmental conditions, can inform practical management of coastal areas in the face of increasing pressure from multiple uses. To date, recreational activities are regulated differently in Mediterranean MPAs (Lloret et al. 2006). In some cases, the entry of scuba divers may still be free, in others it is forbidden, or it is subjected to partial restrictions (e.g., not allowed in the no-take zone of the MPA) or it requires individual registration, sometimes involving the payment of a fee. In the latter case, this approach could induce MPA managers to propose quotas on divers. Recently, because of exponential increase in diving tourism (Hawkins et al. 2005), several studies focused on the impact of diving activity within MPAs, aiming at identifying threshold level of visitation for a given site (Rouphael and Inglis 1997, 2001; Garrahou et al. 1998; Hawkins et al. 2005). However, besides quantifying a value of recreational carrying capacity of a given area, the implementation of a "bottom-up" approach based on the characterization of benthic communities potentially affected by diving activities, contextually evaluating their



vulnerability through indicators of early warning, is critical for effective management.

Impacts from recreational scuba diving on rocky reefs may seem trivial compared with large-scale threats such as overexploitation, species invasion, chemical pollution, and climate change. MPAs have been recently indicated as direct management and conservation actions that have successfully helped in habitat protection (Worm et al. 2006). However, MPAs are increasingly challenged to maintain or increase tourism benefits while protecting the resource, and diving visitation is a small-scale source of impacts (even though increasingly widespread) that can be assessed and managed more easily than large-scale pressures such as climate change. Since recovery can take many decades for long-lived species and complex ecosystems (Lotze et al. 2011) like marine caves, the challenge is a timely reduction of those activities leading to degradation together with an increasing societal awareness leading to the long-term sustainable use of this unique habitat.

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