Impact of a harbour construction on the benthic community of two shallow marine caves

Ettore Nepote, Carlo Nike Bianchi, Carla Morri, Marco Ferrari and Monica Montefalcone*

DiSTAV, Department of Earth, Environment and Life Sciences, University of Genoa, Corso Europa 26, 16132 Genoa, Italy

*Corresponding author: montefalcone@dipteris.unige.it

Abstract

Marine caves are unique and vulnerable habitats, threatened by multiple global and local disturbances. While the effects of climate change on marine caves have already been investigated, no information exists about the effects of local human impacts, such as coastal development, on these habitats. This study investigated the impact of the construction of a touristic harbour on two shallow underwater marine caves in the Ligurian Sea (NW Mediterranean). As a standard methodology for monitoring marine caves does not exist yet, changes over time on the benthic community were assessed adopting two different non-taxonomic descriptors: trophic guilds and growth forms. Harbour construction caused an increase of sediment load within the caves, with a consequent decline of filter feeder organisms. Abundance of small organisms, such as encrusting and flattened sponges, was greatly reduced in comparison to organisms with larger and erect growth forms, such as domed mounds and pedunculated sponges. Our study indicated that growth forms and trophic guilds are effective descriptors for evaluating changes over time in marine caves that could be easily standardised and applied in monitoring plans. In addition, as the harbour construction impacted differently according to the cave topography, the use of a systematic sampling in different zones of an underwater cave is recommended.

Keywords: marine caves, local disturbance, sediment load, non-taxonomic descriptors, growth forms, trophic guilds, Mediterranean Sea.

Introduction

Marine caves are unique and vulnerable ecosystems (Sarà 1976) listed in the EU Habitat Directive 92/43/EEC (habitat type 8330) and in the Barcelona Convention (UNEP-MAP-RAC/SPA 2008; Giakoumi et al. 2013). However, marine caves are not included in the list of those priority habitats needing special conservation efforts. The importance of marine caves is linked to their role in maintaining a high biodiversity along coastal zones (Todaro et al. 2006; Bussotti and Guidetti 2009; Fraschetti et al. 2009), due to their ecological connection (both trophic and at the population level) with other communities of coastal habitats, such as coralligenous and rocky reefs, seagrass beds and sandy bottoms (Harmelin et al. 2003). They also exhibit a connection with the pelagic system, due to water movement, which brings food and propagules into caves (Rastorgueff et al. 2015). Marine caves constitute a naturally fragmented habitat, which can act as a refuge or ecological island (Rastorgueff et al. 2015, and reference therein) and often possess an astonishing ecological and faunal affinity with the deep sea (Boury-Esnault et al. 1993; Bianchi et al. 1996; Janssen et al. 2013). Submerged or semi-submerged marine caves are widely distributed along coastal areas (Rastorgueff et al. 2015), and about 66% of the Mediterranean marine protected areas include marine caves (Abdulla et al. 2008).

Several motile organisms, such as crustaceans, molluscs and coastal fishes, can be found inside marine caves (Harmelin et al. 1985; Bussotti and Guidetti 2009; Bussotti et al. 2015), but the most peculiar resident community is represented by benthic sessile invertebrates such as sponges, ascidians, bryozoans, cnidarians, serpulids and brachiopods (Bianchi 2003). Distribution of these sessile invertebrates within marine caves is dictated by gradients of light and water movement, and vary in terms of species composition, domination and abundance according to the cave topography (Riedl 1966; Morri and Bianchi 2003; Gerovasileiou and

Voultsiadou 2015). For instance, in a blind-end (cul-de-sac) cave with linear topography, the gradients develop regularly along the exterior–interior axis, conforming to the classical zonation scheme: the first zone, at the cave entrance, is dominated by encrusting algae; inside the cave, a second zone is characterised by passive filter feeders; finally, in the terminal zones of the cave only active filter feeders remain (Balduzzi et al. 1989; Morri and Bianchi 2003). In tunnel-shaped caves or in cavities showing different topographies the zonation of communities becomes more complex and may be influenced also by other factors (Parravicini et al. 2010).

Coastal ecosystems are changing under the pressure of global impacts, such as climate warming and ocean acidification (Bianchi et al. 2014; Rodrigues et al. 2015, and references therein). Global impacts may act in a simultaneous way (at the same time) or in an additive way (one after the other) with local impacts such as urbanization, coastal development and overfishing (Airoldi et al. 2005; Rossi 2013; Piggott et al. 2015). Different studies showed the effects of climate change on marine caves (Chevaldonné and Lejeusne 2003; Parravicini et al. 2010; Gerovasileiou and Voultsiadou 2012). However, available information about the effects of local impacts on marine caves only focused on unregulated underwater activities (Di Franco et al. 2010; Guarnieri et al. 2012), whilst no information exist on the effects of coastal constructions on these habitats. This lack of information may represent a serious problem in the view of a correct environmental management (Price et al. 2014). Coastal constructions may cause increase of sediment loads and water turbidity (Davenport and Davenport 2006; Anfuso et al. 2011), which are likely to cause a decline in filter feeders organisms (e.g. sponges), whose filter-feeding apparatus may get clogged by sediments (Bell et al. 2015). Additionally, a coastal construction may alter the hydrodynamic regime and local currents of an area (Anfuso et al. 2011; Ferrari et al. 2014). Variations in the regime of marine currents may change the natural gradients of light penetration and water movement in an underwater cave, i.e. of the two most important factors responsible for the particular environment and for the biological zonation inside the caves (Bianchi and Morri, 1994; Corriero et al. 2000; Martì et al. 2004).

This paper represents the first attempt to investigate the effects of a tourist harbour construction on two underwater marine caves. In order to assess changes over time of the benthic community, two different non-taxonomic descriptors were used, i.e. trophic guilds and growth forms (Parravicini et al. 2010). The former provide information about trophic organization (which depends on light penetration and particulate matter availability), the latter on the degree of ecological confinement (an expression of water exchange and cave topography).

Materials and methods

Study area

The two marine caves are located near Ventimiglia, a city in the Western Liguria, Italy (Fig. 1a). The largest cave is named Grotta Grande (Fig. 1b): it is a semi-submerged marine cave, facing NE, with the entrance at 4 m depth and the terminal part of the cavity at 2.5 m depth with respect to the mean sea level. The cave topography shows an internal subdivision in two sectors after a linear distance of 15 m from the entrance: the western sector continues for about 15 m within the coastal conglomerate, while the eastern sector terminates after about 6 m. The zone of the cave near the entrance has a pebble bottom, whereas, going toward the innermost part of the cave, the bottom is characterized by a layer of fine sediments. The smallest cave is named Grotta Piccola (Fig. 1c): it is a submerged marine cave facing SW, with the entrance between 1.1 and 3.5 m depth. The cave ends after a linear distance of about 20 m from the entrance, diminishing in size at the terminal part with rising chimneys. Like the Grotta Grande cave, the bottom near the entrance is dominated by pebbles but the bottom inside the cave is covered by fine sediment.

The two caves do not have a karstic origin but have formed by a marine erosion process: the coastal rock in which they develop is constituted by the "Conglomerate of Monte Villa", which was deposited during the Middle-Lower Pliocene and is composed of bedded gravelly deposits associated with sandstones related to several deltaic systems (Boni 1984). Therefore, only from a geomorphological point of view, these caves should be better defined as 'pseudocaves' (Eberhard and Sharples 2013) because they are non-karstic but have morphologically karst-like (cavernous morphology) components. However, from an ecological point of view they are not different from other underwater marine caves of the region (Canessa et al. 2014): they will be thus called caves throughout this paper.

At a distance of about 600 m eastward from to two caves is the Roja river (Fig. 2): with a flow rate of 5.92 $m^3 \cdot s^{-1}$, the Roja induces high sedimentation rate and water turbidity in the area (Vacchi et al. 2012). However, as the SW (220-240°N) is the dominant wave direction in Liguria (Fig. 1a), with a fetch greater than 800 km and an offshore wave height of more than 4 m, the sediment flow is mainly directed to the East, and so away from the caves. On the contrary, the SE (130-150°N) wave direction is characterized by a fetch of about 200 km and waves of about 2 m height (Fig. 1a), so that it has a comparatively lower influence on the natural sedimentation rate inside the caves (Vacchi et al. 2012).

Being exactly adjacent, both caves have been exposed to the effects of the construction of a new touristic harbour started in 2010 (Fig. 2a). The project of the harbour covers a water mirror of about 70,000 m^2 with 7 wharfs, which could receive 323 boats. For the harbour construction, a total of 570 t of boulders, 26,000 m^3 of concrete for all wharf structures and

1600 m^3 to support wharf structures, had been used. The seafloor around the new harbour will not have depths greater than 7-8 m. The wharfs are constructed with casting boulders. In 2011, one year after the start of the works, the main wharf was constructed and the central wharf construction begun (Fig. 2b). In 2012, the secondary wharf began (Fig. 2c). In 2013, the construction of the central wharf and of the secondary wharf continued (Fig. 2d) but in 2014 and in 2015 the construction works slowed down. Today (2016), works are still in progress.

Field activity

The two caves were investigated in five distinct periods, in 2010 (before the start of construction works), 2011, 2012, 2013 and 2015, always in summer. Sampling activities were not performed in 2014 because the works were interrupted in that year to be resumed at the beginning of 2015. Unfortunately, lack of other similar underwater marine caves in the area of Ventimiglia determined the impossibility to find appropriate controls to be used in a BACI (before-after control-impact) design (Underwood 1991). Moreover, benthic assemblages of marine caves are highly variable, even at small scales (Bussotti et al. 2006), not allowing the selection of proper controls. Thus, a BA (before-after) design was adopted (Gutperlet et al. 2015). The BA design allows investigating the time x space interaction, whose significance is indicative of different responses to the impact of the stations through time (Guidetti et al. 2014). A photographic sampling technique was applied to study benthic organisms over surfaces of 20 cm x 20 cm (Morri et al. 2003). As a strong gradient exists in both caves, a systematic sampling method was adopted (Krebs 1999, and references therein). The two caves were subdivided into sampling stations regularly spaced from one another, starting from the entrance and moving to the terminal part of the caves: 6 stations in the Grotta Grande (GG-a to GG-f, Fig. 1b) and 3 stations in the Grotta Piccola (GP-a to GP-c, Fig. 1c); 3

random replicated photographs were taken in each station (Corriero et al. 2000; Bussotti et al. 2006). All replicates were taken on the left vertical walls of the caves.

Data analysis

Non-taxonomic descriptors were used to assess change in the sessile community, i.e. trophic guilds and growth forms (Parravicini et al. 2010). Considering the energy sources they exploit, the sessile organisms of the caves were divided in eight trophic guilds: autotrophs (i.e. algae), passive filter feeders (e.g. cnidarians), active ciliate (e.g. serpulids and spirorbids), active ciliate with lophophore (e.g. bryozoans and brachiopods), active pumping sponges, active pumping ascidians, mixotrophic sponges (e.g. *Petrosia ficiformis*, which may host photosynthetic endosymbionts), and mixotrophic algae (e.g. Chrysophyceae, which may exploit organic compounds). Growth forms were used to investigate different strategies of substratum occupation (Jackson 1979; Connell and Keough 1985). Considering the ratio between the height (h) and the radius (r) of the organism, ten different growth forms were recognized: prostrate algae, erect algae, runners, determinate sheets, indeterminate sheets, flattened ($h < r$) mounds, hemispherical ($h = r$) mounds, domed ($h > r$) mounds, vines and pedunculated sponges. Runners and sheets (either determinate or indeterminate) are 2-dimensional, strictly adhering to the substrate; mounds and vines are 3-dimensional, projecting to some extent into the water column and producing higher habitat complexity. Two additional categories were included in the analyses to take into account the abiotic components: bare substrate, and turf and sediment considered together (due to the operational difficulty to separate them during image analysis), both providing an indication of environmental stress (Gatti et al. 2015a; Vannini et al. 2015). On each photograph (18 for the

Grotta Grande and 9 for the Grotta Piccola per each year) the percent cover of all non-taxonomic descriptors was visually estimated.

To assess change over time in the two caves, ecological distances, expressed as the average (± se) Euclidean distance between the 3 photoquadrats of a sampling station in a given year and the centroid of the photoquadrats in 2010, were calculated (Gatti et al. 2015b). Euclidean distance was chosen because it is particularly adequate to assess the measure of dissimilarity between two samples (Anderson et al. 2011). Arcsine $\sqrt{(x/100)}$ transformation was applied to cover data (Legendre and Legendre 1998). Univariate analyses of variance (ANOVAs) were performed to assess significant differences in the most abundant descriptors through time and in those descriptors that appeared after 2010 and that might be a consequence of the impact. The model of the analysis consisted of 2 factors: the factor “station” (fixed, 6 levels in the Grotta Grande, 3 in the Grotta Piccola), which is orthogonal to the factor “year” (random, 5 levels for the two caves). Prior to the analyses, the homogeneity of variance was tested by Cochran’s test and, if necessary, data were appropriately transformed. When a treatment factor was significant, the post-hoc comparison of the means was performed with SNK test. Using the same model, PERMANOVA analyses were also performed. This method analyses the variance of multivariate data explained by one or more explanatory factors and gives *p*-values calculated using all possible permutations (Clarke and Warwick 1994). When a treatment factor was significant, the post-hoc comparison of the means was performed with the pair-wise test.

Starting from the fourth sampling period, in 2013, the terminal zone of the Grotta Piccola resulted occluded by a huge deposit of fine sediment and organic matter, thus preventing the possibility to collect the 3 photographic samples in the station GP-c (see Fig. 6l). These 3 missing samples were operationally considered in the statistical analyses as completely covered by sediment (100%) in 2013 and 2015.

Results

Grotta Grande

PERMANOVA showed that the factor year was significant ($p < 0.01$) considering both growth forms and trophic guilds and the pair-wise comparisons revealed that the sessile community in 2010 was significantly different from all the other years, and that it continuously changed through time (Table 1). No differences were found between the last two periods (2013 *vs* 2015). A significant year × station interaction was found when considering sediment and turf (Table 2), which increased significantly ($p < 0.001$) from 2010 (7 ± 4%) to 2015 (62 ± 21%) in all the stations and became the dominant descriptor in the last two periods (Fig. 3 and Fig. 6), accompanied by a significant decrease ($p < 0.001$) in the bare substrate (Fig. 3), except in the year 2012 when it was not different from 2010 (Table 2). Indeterminate sheets decreased significantly ($p < 0.001$), reducing their cover by almost one third from 2010 to 2015, as well as flattened mounds (Fig. 3), which however showed a significant ($p < 0.001$) lower cover only in 2013 and 2015 (Table 2). Cover of domed mounds did not change over time (Fig. 3). In 2013, pedunculated sponges appeared (Fig. 6) with cover values lower than 10%. The cover of active pumping sponges was higher ($p < 0.001$) in 2010 with respect to other years (Fig. 3 and Table 2). Autotrophs and mixotrophic sponges did not change over time (Fig. 3 and Fig. 6). The temporal drift (i.e. the rate of change) evidenced by ecological distances was the same for trophic guilds and growth forms and all stations denoted the same temporal drift (Fig. 5) experiencing the greatest change between 2010 and 2011 and between 2012 and 2013 (see also Fig. 6). All the stations did not show significant changes from 2013 to 2015.

Grotta Piccola

In the Grotta Piccola a significant year × station interaction was found with PERMANOVA considering both growth forms and trophic guilds, and the pair-wise comparisons indicated that the sessile community in 2010 was significantly different ($p < 0.01$) from 2011, 2012, 2013 and 2015 only in the station GP-c, i.e. the innermost station (Table 1). Also in this cave, the cover of sediment and turf increased significantly ($p < 0.01$) from 2010 (29 ± 29%) to 2015 (88 ± 14%) in all the stations, becoming the dominant descriptor in the last three periods, accompanied by a decrease in the bare substrate especially in the station GP-c ($p < 0.001$) (Fig. 4 and Table 3, and see also Fig. 7). Indeterminate sheets decreased significantly ($p < 0.01$) from 2012 and nearly disappeared in the subsequent sampling periods (Fig. 4 and Table 3). Cover of prostrate and erect algae did not change over time (Fig. 4 and Fig. 7). Similarly to Grotta Grande, when considering trophic guilds only active pumping sponges showed a significant change through time having a higher cover ($p < 0.01$) in 2010, whilst autotrophs did not change (Fig. 4 and Table 3). In 2015, mixotrophic algae (Chrysophyceae) appeared for the first time (Fig. 4 and Fig. 7). The temporal drift evidenced by ecological distances was the same for trophic guilds and growth forms (Fig. 5) also in this cave. According to PERMANOVA, stations GP-a and GP-b did not show significant temporal changes, whilst station GP-c showed the greatest changes between 2010 and 2013.

Discussion

The benthic community of the two underwater marine caves changed dramatically following the touristic harbour construction. The cover of sessile organisms declined, while sediment and turf increased on the walls of the caves. The natural hydrodynamic regime in the study area do not explain the high sedimentation rates found in the two caves, as the littoral drift flows from West to East (Vacchi et al. 2012) and the sediment originating from the close Roja river follows this direction. Already after few months (in 2011) from the start of the works for the harbour construction, the amount of sediment (as estimated on the photographs) has increased by 4-fold in the Grotta Grande and by 3-fold in the Grotta Piccola, and after 5 years it was 6-fold and 4-fold higher in the two caves, respectively. This huge amount of sediment covered all the bare substrate on the walls of the caves and caused a decline of sessile organisms (Fig. 6). As expected, encrusting and flattened growth forms, such as indeterminate sheets and flattened mounds, were the most affected, whilst those forms rising up from the substrate, such as domed mounds, did not decline significantly over time (Fig. 6c, e). Encrusting and flattened organisms are favourite by good water exchanges within submerged caves (Bell and Barnes 2000; Bussotti et al. 2006; Parravicini et al. 2010). On the contrary, erect forms are more difficultly covered by sediments (Bell and Barnes 2000). Morphology of sessile organism was thus an effective descriptor to assess the effects of the harbour construction. Another clear sign of the sediment impact was the metamorphosis in the growth form experienced by the sponges of the genus *Petrosia* and *Chondrosia*, whom progressively modified their flattened mounds to a pedunculated morphology (Fig. 6f) - a growing strategy to counteract the silting (Bell and Barnes 2000). This particular growth form is also favoured in environments with low water exchanges because it allows a greater efficiency in the elimination of catabolites (Morri and Bianchi 2003).

Taking into account the feeding strategy, active pumping sponges showed the greatest decline. High sedimentation rates are known to cause clogging of their feeding apparatus, which may lead to the death of the organism (Bell et al. 2015). Autotrophs exhibited no decline in the caves (Fig. 6a, b), and this is likely related with their position restricted only at the entrance of the cave, which is subjected to less extreme environmental conditions (Morri and Bianchi 2003). Although increased levels of sedimentation in the caves are likely to enhance water turbidity, mixotrophic organisms, such as the sponge *Petrosia ficiformis*, did not lose their autotrophic endosymbionts over time, maybe because the vicinity of the caves to the sea surface allowed a sufficient light intensity to be maintained (Fig. 6d, e).

The increased sedimentation within the two caves is likely to be due to a combined effect of constructional material released in the water and changes in the natural hydrodynamic regime of the area due to the realization of the primary and secondary wharfs, this latter being next to the Grotta Piccola (see Fig. 2). If the caves were exposed to direct effect of waves and currents before the harbour construction, especially to waves coming from the SE direction (Vacchi et al. 2012), the wharf structures acted as an artificial barrier slowing water exchange within the caves and favouring sediment deposition in all the area around the secondary wharf (Martì et al. 2004). In addition, as the secondary wharf interrupted the natural littoral drift, thus favouring further sediment deposition, starting from 2013 the terminal part of the Grotta Piccola has been completely occluded by a plug of fine sediments and organic matter deriving from seagrass leaves decomposition (Fig. 6l, m). It may also be hypothesised that the continuous deposition of sediments in the area will require the dredging of the harbour in the future, a further source of disturbance for the two caves in the years to come (Clarke and Tully 2014).

The anoxic environment caused by organic matter deposition and low water exchange favoured the development of sulphur bacteria on the vault and on the walls of the Grotta Piccola (Fig. 6 h to n), which act as primary producers fixing carbons through chemosynthesis (Airoldi and Cinelli 1996; Herbet et al. 2005). The appearance of degrading bacteria in a polluted environment has been recognized as a strategy for enhancing the clean-up of sediments (Barbato et al. 2016, and references therein). In 2015, mixotrophic Chrysophyceae (Fig. 6i, n) appeared in the Grotta Piccola, which photosynthetically produce dissolved organic carbon that may support the metabolism of sulphur bacteria (Das et al. 2009). Metabolic activity of both sulphur bacteria and Chrysophyceae could help consuming the wide deposit of organic matter on the floor of this cave in the future (Airoldi and Cinelli 1996).

All the non-taxonomic descriptors we adopted, growth forms and trophic guilds, acted as effective indicators of the benthic community alteration within the marine caves. In particular, the morphology of sessile organisms seems to be more indicative of both local impacts and global change effects than the feeding strategy (Parravicini et al. 2010); morphology is also a more simple descriptor to be employed and identified (Bell and Barnes 2001; Bell 2007; Schonberg and Fromont 2013). The use of growth forms as a main descriptor in monitoring programs of marine caves is thus encouraged. The significant time × space interaction we often found evidenced the different response to the impact of the various zones along the caves. Stations positioned at the entrance and in the first part of the caves changed less than stations positioned in the terminal parts of the caves, i.e. in the most confined zone (Bianchi and Morri 1994). This result, also confirmed by other studies focusing on the motile fauna of marine caves (Bussotti and Guidetti 2009; Navarro-Barranco et al. 2015) evidences the adequacy of using a systematic sampling in environments characterized by strong ecological gradients, as recommended by Krebs (1999).

The temporal drift of the benthic community slowed down after 2013, when the works for harbour construction had been temporarily interrupted. Alterations of hydrodynamic regime and sediment load should be always considered in all procedures of environmental impact assessment on marine caves (Feng et al. 2015). The poor resilience of marine caves (Parravicini et al. 2010) induces a pessimistic prospective for the two caves, especially for the

Grotta Piccola. Engineering initiatives aiming at reducing the sediment dispersion during and following coastal constructions should be mandatory to help preserving fragile and precious ecosystems such as underwater caves and other marine coastal habitats.

.

References

Abdulla A, Gomei M, Maison É, Piante C, 2008. Status of Marine Protected Areas in the Mediterranean Sea. IUCN, Málaga, and WWF, Paris, 152 pp.

Airoldi L, Abbiati M, Beck MW, Hawkins SJ, Jonsson PR, Martin D, Moschella PS, Sundelöf A, Thompson RC, Aberg P, 2005. An ecological perspective on the deployment and design of low-crested and other hard coastal defence structures. Ecological Engineering 52, 1073-1087.

Airoldi L, Cinelli F, 1996. Variability of fluxes of particulate material in a submarine cave with chemolithoautotrophic inputs of organic carbon. Marine Ecology Progress Series 139, 205-217.

Anderson MJ, Crist TO, Chase JM, Vellend M, Inouye BD, Freestone AL, Sanders NJ, Cornell HV, Comita LS, Davies KF, Harrison SP, Kraft NJB, Stegen JC, Swenson NG, 2011. Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecology Letters 14 (1), 19-28.

Anfuso G, Pranzini E, Vitale G, 2011. An integrated approach to coastal erosion problems in northern Tuscany (Italy): littoral morphological evolution and cell distribution. Geomorphology 129, 204-214.

Balduzzi A, Bianchi CN, Boero F, Cattaneo Vietti R, Pansini M, Sarà M, 1989. The suspension-feeder communities of a Mediterranean sea cave. Scientia Marina 53 (2-3): 387-395.

Barbato M, Mapelli F, Magagnini M, Chouaia B, Armeni M, Marasco R, Crotti E, Daffonchio D, Borin S, 2016. Hydrocarbon pollutants shape bacterial community assembly of harbour sediments. Marine Pollution Bulletin, in press.

Bell JJ, 2007. The use of volunteers for conducting sponge biodiversity assessments and monitoring using a morphological approach on Indo-Pacific coral reefs. Aquatic Conservation: Marine and Freshwater Ecosystems 17, 133-145.

Bell JJ, Barnes DKA, 2000. The influence of bathymetry and flow regime upon the morphology of sublittoral sponge communities. Journal of Marine Biological Association of the UK 80, 707-718.

Bell JJ, Barnes DKA, 2001. Sponge morphological diversity: a qualitative predictor of species diversity? Aquatic Conservation: Marine and Freshwater Ecosystems 11, 109-121.

Bell JJ, McGrath E, Biggerstaff A, Bates T, Bennett H, Marlow J, Shaffer M, 2015. Sediment impacts on marine sponges. Marine Pollution Bulletin 94, 5-13.

Bianchi CN, 2003. Flora and fauna: general outline and perspectives. In: Cicogna F, Bianchi CN, Ferrari G, Forti P (Eds.), Sea caves: fifty years of research in Italy. Ministero dell’Ambiente e della Tutela del Territorio, Roma, pp. 137-146.

Bianchi CN, Cattaneo-Vietti R, Cinelli F, Morri C, Pansini M, 1996. The biological study of the submarine caves of the Mediterranean: present knowledge and perspectives. Bollettino dei Musei e degli Istituti Biologici dell’Università di Genova 60-61 (1994-95), 41-69.

Bianchi CN, Corsini-Foka M, Morri C, Zenetos A, 2014. Thirty years after: dramatic change in the coastal marine ecosystems of Kos Island (Greece), 1981-2013. Mediterranean Marine Science 15 (3), 482-497.

Bianchi CN, Morri C, 1994. A bionomical comparative study of some submerged marine caves: definition of a confinement scale. Memorie dell’Istituto Italiano di Speleologia 6 (s. II), 107-123.

Boni A, 1984. Il Pliocene al margine delle Alpi Liguri. Memorie Società Geologica Italiana 28, 114-125.

Boury-Esnault N, Harmelin JG, Vacelet J, 1993. Les abysses méditerranéennes à vingt mètres de profondeur? La Recherche 24 (256) suppl., 848-851.

Bussotti S, Di Franco A, Francour P, Guidetti P, 2015. Fish assemblages of Mediterranean marine caves. Plos One 10 (4), e0122632.

Bussotti S, Guidetti P, 2009. Do Mediterranean fish assemblages associated with marine caves and rocky cliffs differ? Estuarine Coastal and Shelf Science 81, 65-73.

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

Canessa M, Montefalcone M, Cánovas Molina A, Coppo S, Diviacco G, Bavestrello G, Morri C, Bianchi CN, 2014. Submerged marine caves of Liguria: updating the knowledge. In: Langar H, Bouafif C, Ouerghi A (Eds.), Proceedings of the 1st Mediterranean symposium on the conservation of dark habitats. UNEP/MAP–RAC/SPA, Tunis, pp. 27-32.

Chevaldonné P, Lejeusne C, 2003. Regional warming induced species shift in NW Mediterranean marine caves. Ecology Letters 6, 371-379.

Clarke S, Tully O, 2014. BACI monitoring of effects of hydraulic dredging for cockles on intertidal benthic habitats of Dundalk Bay, Ireland. Journal of the Marine Biological Association of the UK 94, 1451-1464.

Clarke KR, Warwick RM, 1994. Change in marine communities: an approach to statistical analysis and interpretation. Natural Environment Research Council, UK, 144 pp.

Connell JH, Keough MJ, 1985. Disturbance and patch dynamics of subtidal marine animals on hard substrata. In: Pickett STA, White PS (Eds.), The ecology of natural disturbance and patch dynamics. Academic Press, San Diego, 472 pp.

Corriero G, Liaci LS, Ruggiero D, Pansini M, 2000. The sponge community of a semi-submerged Mediterranean cave. Marine Ecology 21, 85-96.

Corsini S, Inghilesi R, Franco L, Piscopia R, 2006. Italian waves atlas. APAT e Università degli Studi di Roma Tre, Roma, 152 pp.

Das BK, Roy A, Koschorreck M, Mandal SM, Wendt-Potthoff K, Bhattacharya J, 2009. Occurrence and role of algae and fungi in acid mine drainage environment with special reference to metals and sulphate immobilization. Water Research 43, 883-894.

Davenport J, Davenport JL, 2006. The impact of tourism and personal leisure transport on coastal environments: A review. Estuarine Coastal Shelf Science 67, 280-292.

Di Franco A, Ferruzza G, Baiata P, Chemello R, Milazzo M, 2010. Can recreational scuba divers alter natural gross sedimentation rate? A case study from a Mediterranean deep cave. Journal of Marine Science 67, 871-874.

Eberhard R, Sharples C, 2013. Appropriate terminology for karst-like phenomena: the problem with 'pseudokarst'. International Journal of Speleology 42, 109-113.

Feng L, He J, Ai J, Sun X, Bian F, Zhu X, 2015. Evaluation for coastal reclamation feasibility using a comprehensive hydrodynamic framework: A case study in Haizhou Bay. Marine Pollution Bulletin 100, 182-190.

Ferrari M, Cabella R, Berriolo G, Montefalcone M, 2014. Gravel sediment bypass between contiguous littoral cells in the NW Mediterranean Sea. Journal of Coastal Research 30, 183-191.

Fraschetti S, D'Ambrosio P, Micheli F, Pizzolante F, Bussotti S, Terlizzi A, 2009. Design of marine protected areas in a human-dominated seascape. Marine Ecology Progress Series 375, 13-24.

Gatti G, Bianchi CN, Morri C, Montefalcone M, Sartoretto S, 2015a. Coralligenous reefs state along anthropized coasts: application and validation of the COARSE index, based on a rapid visual assessment (RVA) approach. Ecological Indicators 52, 567-576.

Gatti G, Bianchi CN, Parravicini V, Rovere A, Peirano A, Montefalcone M, Massa F, Morri C, 2015b. Ecological change, sliding baselines and the importance of historical data: lessons from combining observational and quantitative data on a temperate reef over 70 years. Plos One 10 (2), e0118581.

Gerovasileiou V, Voultsiadou E, 2012. Marine caves of the Mediterranean sea: a sponge biodiversity reservoir within a biodiversity hotspot. Plos One 7, e39873.

Gerovasileiou V, Voultsiadou E, 2015. Sponge diversity gradients in marine caves of the eastern Mediterranean. Journal of Marine Biological Association of the UK 96, 407-416.

Guarnieri G, Terlizzi A, Bevilacqua S, Fraschetti S, 2012. Increasing heterogeneity of sensitive assemblages as a consequence of human impact in submarine caves. Marine Biology 159, 1155-1164.

Guidetti P, Parravicini V, Morri C, Bianchi CN, 2014. Against nature? Why ecologists should not diverge from natural history. Vie et Milieu - Life and Environment 64 (1), 1-8.

Gutperlet R, Capperucci RM, Bartholomä A, Kröncke I, 2015. Benthic biodiversity changes in response to dredging activities during the construction of a deep-water port. Marine Biodiversity 45, 819-839.

Harmelin JG, Boury-Esnault N, Fichez R, Vacelet J, Zibrowius H, 2003. Peuplements de la grotte sous-marine de l'île de Bagaud (parc national de Port-Cros, France, Méditerranée). Scientific Report Port-Cros National Park 19, 117-134.

Harmelin JG, Vacelet J, Vasseur P, 1985. Les grottes sous-marines obscures: un milieu extrême et un remarquable biotope refuge. Tethys 11, 214-229.

Herbert RA, Ranchou-Peyruse A, Duran R, Guyoneaud R, Schwabe S, 2005. Characterization of purple sulphur bacteria from the South Andros Black Hole cave system: highlights taxonomic problems for ecological studies among the genera *Allochromatium* and *Thiocapsa*. Environmental Microbiology 7, 1260-1268.

Jackson JBC, 1979. Morphological strategies of sessile animals. In: Larwood G, Roser BR (Eds.), Biology and systematic of colonial organisms. Academic Press, London, pp. 499-555.

Janssen A, Chevaldonné P, Martínez Arbizu P, 2013. Meiobenthic copepod fauna of a marine cave (NW Mediterranean) closely resembles that of deep-sea communities. Marine Ecology Progress Series 479, 99-113.

Krebs CJ, 1999. Ecological methodology, 2nd edition. Addison-Wesley Longman, Menlo Park, California, 620 pp.

Legendre P, Legendre L, 1998. Numerical ecology, 2nd English edition. Elsevier, Amsterdam, 853 pp.

Martì R, Uriz MJ, Ballesteros E, Turon X, 2004. Benthic assemblages in two Mediterranean caves: species diversity and coverage as a function of abiotic parameters and geographic distance. Journal of Marine Biological Association of the UK 84, 557-572.

Morri C, Bianchi CN, 2003. Biological zonation. In: Cicogna F, Bianchi, CN, Ferrari G, Forti P (Eds.), Sea caves: fifty years of research in Italy. Ministero dell'Ambiente e della tutela del Territorio, Roma, pp. 257-265.

Morri C, Bussotti S, Guidetti P, Bianchi CN, 2003. Sampling and measurement techniques. In: Cicogna F, Bianchi CN, Ferrari G, Forti P (Eds.), Sea caves: fifty years of research in Italy. Ministero dell'Ambiente e della Tutela del Territorio, Roma, pp. 337-345.

Navarro-Barranco C, Guerra-García JM, Sánchez-Tocino L, Ros M, Florido M, García-Gómez JC, 2015. Colonization and successional patterns of the mobile epifaunal community along an environmental gradient in a marine cave. Marine Ecology Progress Series 521, 105-115.

Parravicini V, Guidetti P, Morri C, Montefalcone M, Donato M, Bianchi CN, 2010. Consequences of sea water temperature anomalies on a Mediterranean submarine cave ecosystem. Estuarine Coastal Shelf Science 86, 276-282.

Piggott JJ, Townsend CR, Matthaei CD, 2015. Reconceptualizing synergism and antagonism among multiple stressors. Ecology and Evolution 5 (7), 1538-1547.

Price ARG, Jaoui K, Pearson K, Jeudy de Grissac A, 2014. An alert system for triggering different levels of coastal management urgency: Tunisia case study using rapid environmental assessment data. Marine Pollution Bulletin 80, 88-96.

Rastorgueff PA, Bellan-Santini D, Bianchi CN, Bussotti S, Chevaldonné P, Guidetti P, Harmelin JG, Montefalcone M, Morri C, Perez T, Ruitton S, Vacelet J, Personnic S, 2015. An ecosystem-based approach to evaluate the ecological quality of Mediterranean undersea caves. Ecological Indicators 54, 137-152.

Riedl R, 1966. Biologie der Meereshöhlen. Paul Parey, Hamburg, 602 pp.

Rodrigues LC, van den Bergh JCJM, Loureiro ML, Nunes PALD, Rossi S, 2015. The cost of Mediterranean Sea warming and acidification: A choice experiment among scuba divers at Medes Islands, Spain. Environmental Resource Economy 63, 289-311.

Rossi S, 2013. The destruction of the 'animal forests' in the oceans: Towards an over simplification of the benthic ecosystems. Ocean & Coastal Management 84, 77-85.

Sarà M, 1976. Il popolamento delle grotte marine: interesse di una salvaguardia. Pubblicazioni della Stazione Zoologica di Napoli 40, 502-505.

Schönberg CHL, Fromont J, 2013. Functional sponge morphologies. In: Edwards L. (Ed.) CATAMI classification scheme for scoring marine biota and substrata in underwater imagery. Technical Report, http://catami.org/classification.

Todaro MA, Leasi F, Bizzarri N, Tongiorgi P, 2006. Meiofauna densities and gastrotrich community composition in a Mediterranean Sea cave. Marine Biology 149, 1079-1091.

Underwood AJ, 1991. Beyond BACI: Experimental designs for detecting human environmental impacts on temporal variations in natural populations. Australian Journal of Marine and Freshwater Research 42, 569-587.

UNEP-MAP-RAC/SPA, 2008. Action plan for the conservation of the coralligenous and other calcareous bio-concretions in the Mediterranean Sea. Ed. RAC/SPA, Tunis, 21 pp.

Vacchi M, Montefalcone M, Bianchi CN, Morri C, Ferrari M, 2012. Hydrodynamic constraints to the seaward development of *Posidonia oceanica* meadows. Estuarine Coastal and Shelf Science 97, 58-65.

Vannini C, Volpi M, Lardicci C, 2015. A multi size-level assessment of benthic marine communities in a coastal environment: Are they different sides of the same coin? Plos One 10 (6), e0129942.

Figure captions and tables headings

Figure 1. Geographic location of the study area with the relative annual wave climate (data from Corsini et al. 2006, modified). H_{S0} is the mean annual significant offshore wave height (m) recorded by the La Spezia buoy (43°55'41.99" N; 09°49'36.01" E) (a); topography of the Grotta Grande (b) and of the Grotta Piccola (c), redrawn from “Catasto delle Grotte della Liguria DSL/SSI” and with the position of the sampling stations (GG-a to GG-f in the Grotta Grande, GP-a to GP-c in the Grotta Piccola).

Figure 2. Progress of construction works of the Ventimiglia touristic harbour in 2010 (a), 2011 (b), 2012 (c), 2013 (d). Aerial imagery from Google Earth. Locations of the two underwater caves and the main directions followed by littoral drift and Roja river flow are reported.

Figure 3. Grotta Grande: change over time of the mean (± sd) cover data (%) of the most abundant growth forms (turf and sediment, bare substrate, indeterminate sheets, domed mounds, flattened mounds) and trophic guilds (active pumping sponges, autotrophs, mixotrophic sponges). n.a. = data not available.

Figure 4. Grotta Piccola: change over time of the mean (± sd) cover data (%) of the most abundant growth forms (turf and sediment, prostrate algae, indeterminate sheets, erect algae, bare substrate) and trophic guilds (autotrophs, mixotrophic algae, active pumping sponges). n.a. = data not available.

Figure 5. Ecological distance, expressed as mean Euclidean distance (ED) (± se), of the stations GG-a, GG-b, GG-c, GG-d, GG-e, GG-f in the Grotta Grande and GP-a, GP-b and GP-c in the Grotta Piccola.

Figure 6. Representative photographic samples from Grotta Grande. Station GG-a, at the entrance of the cave, in 2010, 2011 and 2015; station GG-b, with the mixotrophic sponge *Petrosia ficiformis*, and station GG-c in the intermediate zone of the cave in 2010, 2011 and in 2015: in this latter year sponges with a pedunculated form appeared for the first time and a thick layer of sediment covered all encrusting and flattened growth forms but not domed mounds; station GG-e, in the terminal zone of the cave, in 2010, 2011 and 2015. Photos by Eugenio Beccornia (2010) and Monica Montefalcone (remaining years).

Figure 7. Representative photographic samples from Grotta Piccola. Station GP-a, at the entrance of the cave, in 2010, 2011 and 2015; station GP-b, in the intermediate zone of the cave, in 2010, 2011 and 2015: in this latter year sponges with a pedunculated form appeared for the first time; station GP-c, in the terminal zone of the cave, in 2010, 2011 and 2015, where the walls have been completely covered by turf, sediment, mixotrophic algae (Chrysophyceae) and sulphur bacteria; terminal zone of the cave in 2015 that was occluded by the deposition of fine sediment and organic matter deriving from seagrass leaves decomposition that caused the floor rising: wall covered by fine sediment (on the left), floor covered by fine sediment and organic matter that favoured sulphur bacteria development (in the centre) and vault (on the right) covered by mixotrophic algae and sulphur bacteria. Photos by Eugenio Beccornia (2010) and Monica Montefalcone (remaining years).

Table 1. Results of PERMANOVA analyses performed on growth forms and trophic guilds datasets from Grotta Grande and Grotta Piccola. ye = year; st = station. * = $p<0.01$; ** = $p<0.001$; n.s. = not significant.

Table 2. Results of ANOVA analyses performed on turf and sediment, bare substrate, indeterminate sheet, flattened mounds and active pumping sponges in the Grotta Grande. ye = year; st = station. * = $p<0.01$; ** = $p<0.001$; n.s. = not significant.

Table 3. Results of ANOVA analyses performed on turf and sediment, bare substrate, indeterminate sheet, flattened mounds and active pumping sponges in the Grotta Piccola. ye = year; st = station. * = $p<0.01$; ** = $p<0.001$; n.s. = not significant.

Table 1.

Grotta Grande

		Growth forms			Trophic guilds		
Source	**df**	**SS**	**MS**	***p* (perm)**	**SS**	**MS**	***p* (perm)**
ye	4	35872	8968.1	0.001[a]	34438	8609.6	0.001[b]
st	5	29523	5904.7	0.001	31520	6304	0.001
ye×st	20	12108	605.4	0.053	7434	371.7	0.169
Res	60	26057	434.3		17980	299.7	
Total	89	1.0356 E5			91373		

Pair-wise test:
[a] ye: 2010 *vs* 2011**, 2012**, 2013**, 2015**
2011 *vs* 2012*, 2013**, 2015**
2012 *vs* 2013**, 2015**
2013 *vs* 2015 n.s.

Pair-wise test:
[b] ye: 2010 *vs* 2011**, 2012**, 2013**, 2015**
2011 *vs* 2012*, 2013**, 2015**
2012 *vs* 2013**, 2015**
2013 *vs* 2015 n.s.

Grotta Piccola

		Growth forms			Trophic guilds		
Source	**df**	**SS**	**MS**	***p* (perm)**	**SS**	**MS**	***p* (perm)**
ye	4	19104	4776	0.001	19918	4979.6	0.001
st	2	16903	8451.5	0.001	14599	7299.4	0.001
ye×st	8	9543	1192.9	0.003[c]	8471.5	1058.9	0.001[d]
Res	30	12503	416.8		8680.4	289.4	
Total	44	58054			51669		

Pair-wise test:
[c] ye x st:
st GP-c: 2010 *vs* 2011*, 2012*, 2013*, 2015*

Pair-wise test:
[d] ye x st:
st GP-c: 2010 *vs* 2011*, 2012*, 2013*, 2015*

Table 2.

		Turf and sediment				Bare substrate			
Source	**df**	**SS**	**MS**	**F**	***p***	**SS**	**MS**	**F**	***p***
ye	4	43393.1	10848.2	47.48	0.000	172.8	43.2	30.6	0.000[b]
st	5	6251.2	1250.2	2.37	0.076	13.8	2.7	2.2	0.081
ye×st	20	10542.8	527.1	2.31	0.007[a]	24.5	1.2	0.8	0.621
Res	60	13707.4	228.4			84.5	1.4		
Total	89	73894.6				295.7			
Cochran's C-test		n.s.				C= 0.2967* Transformation = arcsine			

		Indeterminate sheet				Flattened mounds			
Source	**df**	**SS**	**MS**	**F**	***p***	**SS**	**MS**	**F**	***p***
ye	4	5328.2	1332	16.4	0.000[c]	2224.1	556	3.6	0.009[d]
st	5	825.1	165	1.9	0.132	1730.9	346	1.5	0.222
ye×st	20	1733.6	86.6	1	0.400	4516.6	226	1.5	0.112
Res	60	4871.2	81.1			9060.8	151		
Total	89	12758.2				17532.6			
Cochran's C-test		n.s.				n.s			

		Active pumping sponges			
Source	**df**	**SS**	**MS**	**F**	***p***
ye	4	14195.6	3548.9	16.9	0.000[e]
st	5	9830.5	1966.1	8.2	0.000
ye×st	20	4773.6	238.6	1.1	0.33
Res	60	12573.7	209.5		
Total	89	41373.6			
Cochran's C-test		n.s.			

SNK test:

[a] ye x st:

- st GG-a: 2010<2013**
- st GG-b: 2010<2011**, <2013*
- st GG-c: 2010<2011**, <2013**, <2015**
- st GG-d: 2010<2011**, <2012**, <2013**, <2015**
- st GG-e: 2010<2011**, <2012**, <2013**, <2015**
- st GG-f: 2010<2013**, <2015**

[b] ye: 2010>2011**, >2013**, >2015**

[c] ye: 2010>2011**, >2012**, >2013**, >2015**

[d] ye: 2010>2013**, >2015**

[e] ye: 2010>2011**, >2012**, >2013**, >2015**

Table 3.

		Turf and sediment				Bare substrate			
Source	**df**	**SS**	**MS**	**F**	***p***	**SS**	**MS**	**F**	***p***
ye	4	27129.9	6782.4	27.47	0.000	5835.8	1458.9	16.1	0.000
st	2	1210	605	1.02	0.404	5822.8	2911.4	3.5	0.083
ye×st	8	4756.7	594.5	2.41	0.039[a]	6657.6	832.2	9.2	0.000[b]
Res	30	7406.2	246.8			2712.4	90.4		
Total	44	40502.9				21028.8			
Cochran's C-test	n.s.					C= 0.7061*			

		Indeterminate sheet				Flattened mounds			
Source	**df**	**SS**	**MS**	**F**	***p***	**SS**	**MS**	**F**	***p***
ye	4	16.6	4.1	8.08	0.000[c]	108.1	27	1.9	0.121
st	2	9.6	4.8	9.79	0.007	87.2	43.6	5.2	0.032
ye×st	8	3.9	0.4	0.96	0.487	66.5	8.3	0.6	0.770
Res	30	15.4	0.5			416.6	13.8		
Total	44	45.6				678.5			
Cochran's C-test	C= 0.5006* Transformation = arcsine					C= 0.6096*			

		Active pumping sponges			
Source	**df**	**SS**	**MS**	**F**	***p***
ye	4	2152.1	538	9.42	0.000[d]
st	2	536.8	268.4	2.15	0.174
ye×st	8	1000.9	125.1	2.19	0.053
Res	30	1713.6	57.1		
Total	44	5403.5			
Cochran's C-test	n.s.				

SNK test:

[a] ye x st:
- st GG-a: 2010<2011*, <2013**, <2015*
- st GG-b: 2010<2012**, <2013**, <2015**
- st GG-c: 2010<2011**, <2012**, <2013**, <2015**

[b] ye x st:
- st GG-a: n.s.
- st GG-b: n.s.
- st GG-c: 2010>2011**, >2012**, >2013**, >2015**

[c] ye: 2010>2012*, >2013*,>2015*

[d] ye: 2010>2012*, >2013*,>2015*

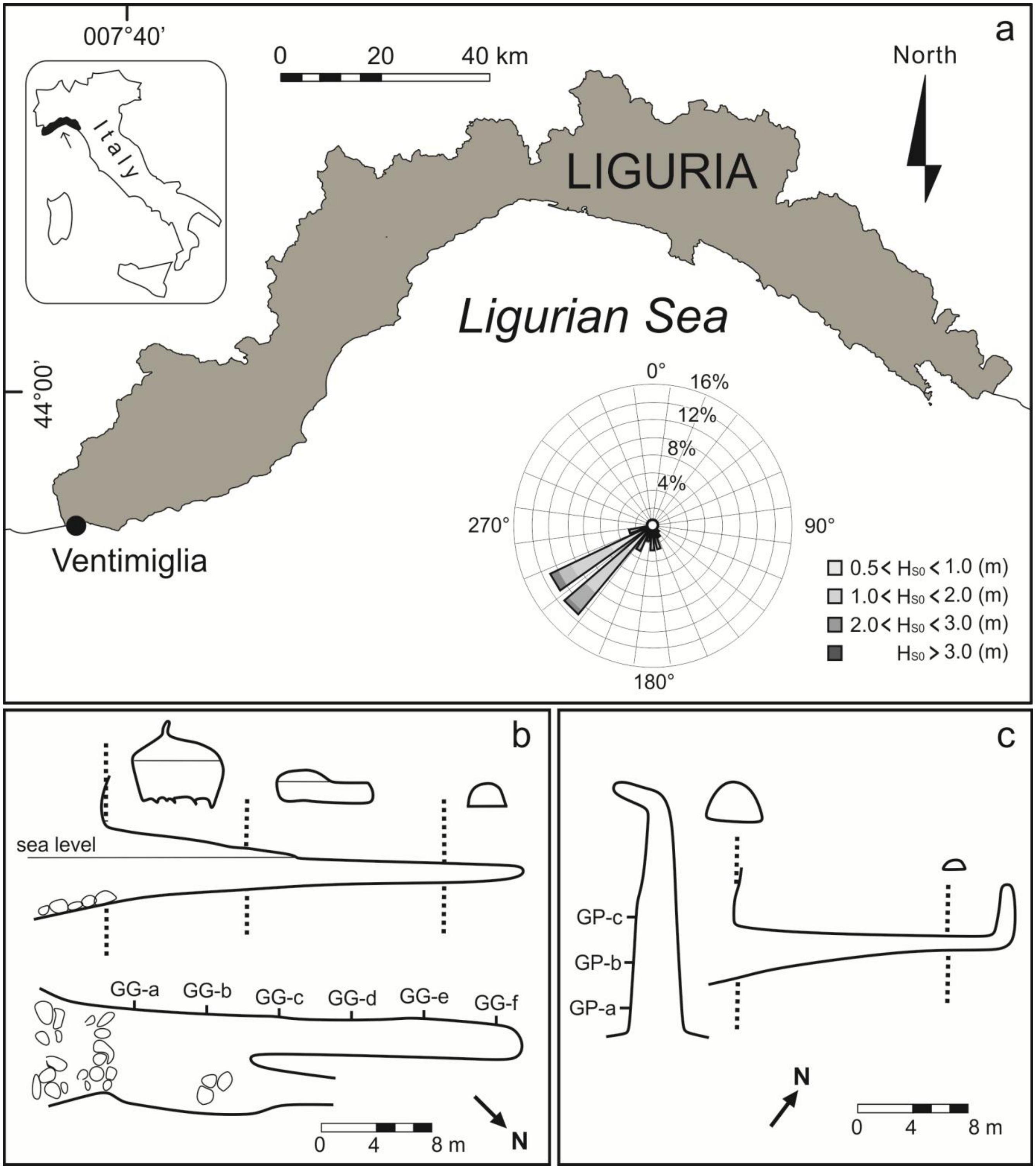

a
007°40’
0
20
40 km
North
Italy
LIGURIA
Ligurian Sea
44°00’
Ventimiglia
0°
16%
12%
8%
4%
270°
90°
180°
☐ 0.5< Hs0 < 1.0 (m)
☐ 1.0< Hs0 < 2.0 (m)
▨ 2.0< Hs0 < 3.0 (m)
■ Hs0 > 3.0 (m)
b
sea level
GG-a
GG-b
GG-c
GG-d
GG-e
GG-f
0
4
8 m
N
c
GP-c
GP-b
GP-a
N
0
4
8 m

Figure 1

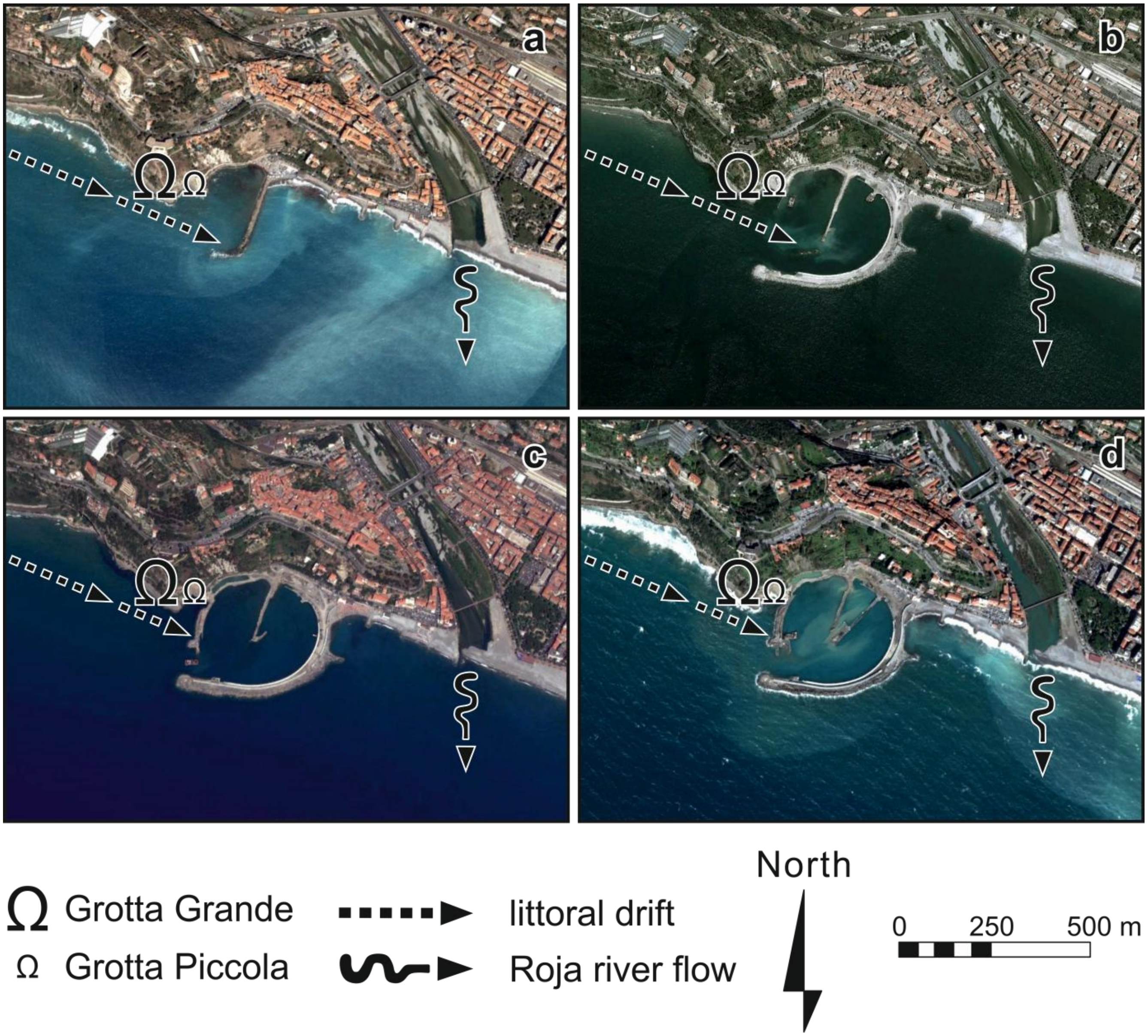

a
b
c
d
Ω Grotta Grande
Ω Grotta Piccola
littoral drift
Roja river flow
North
0
250
500 m

Figure 2

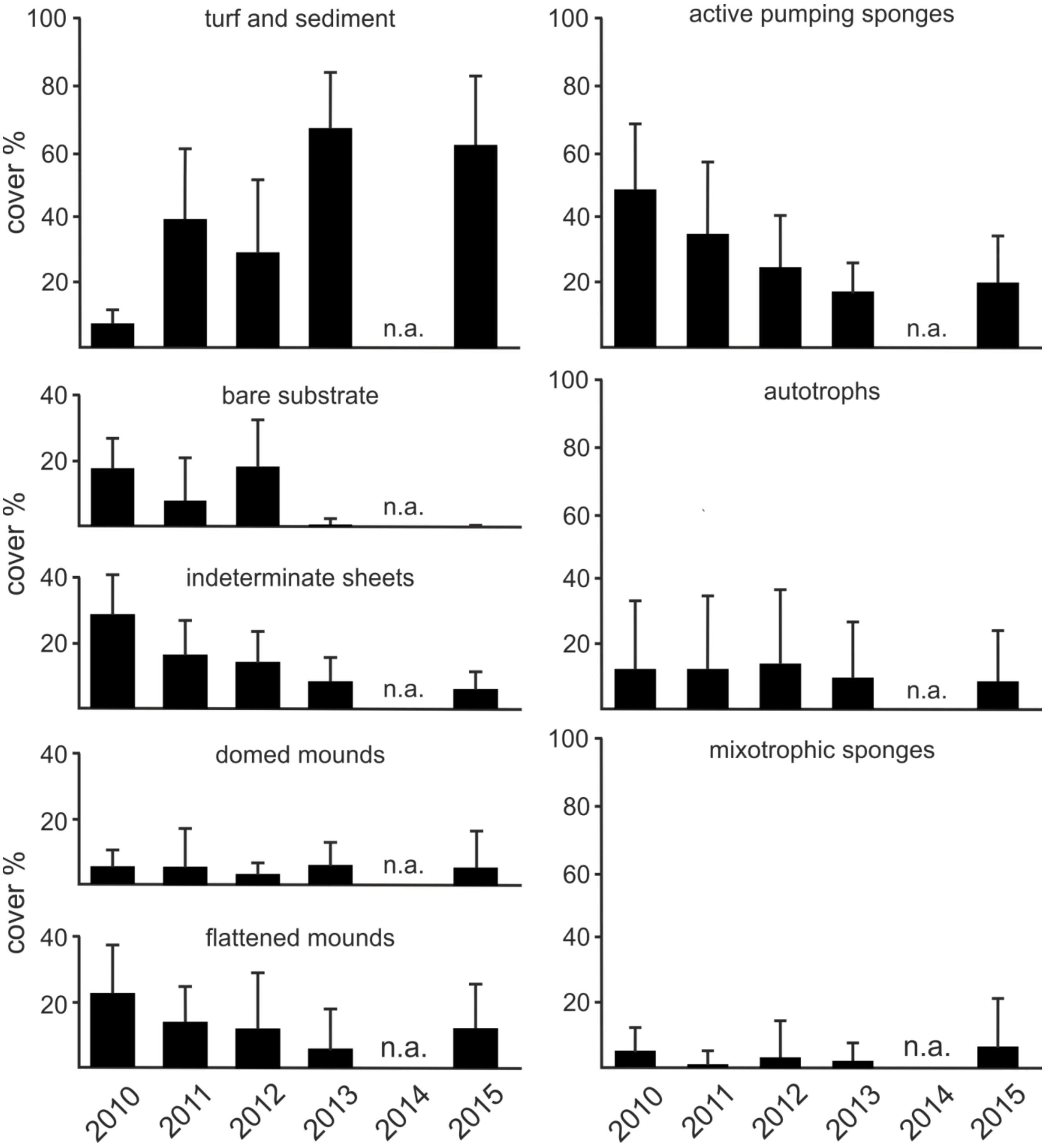
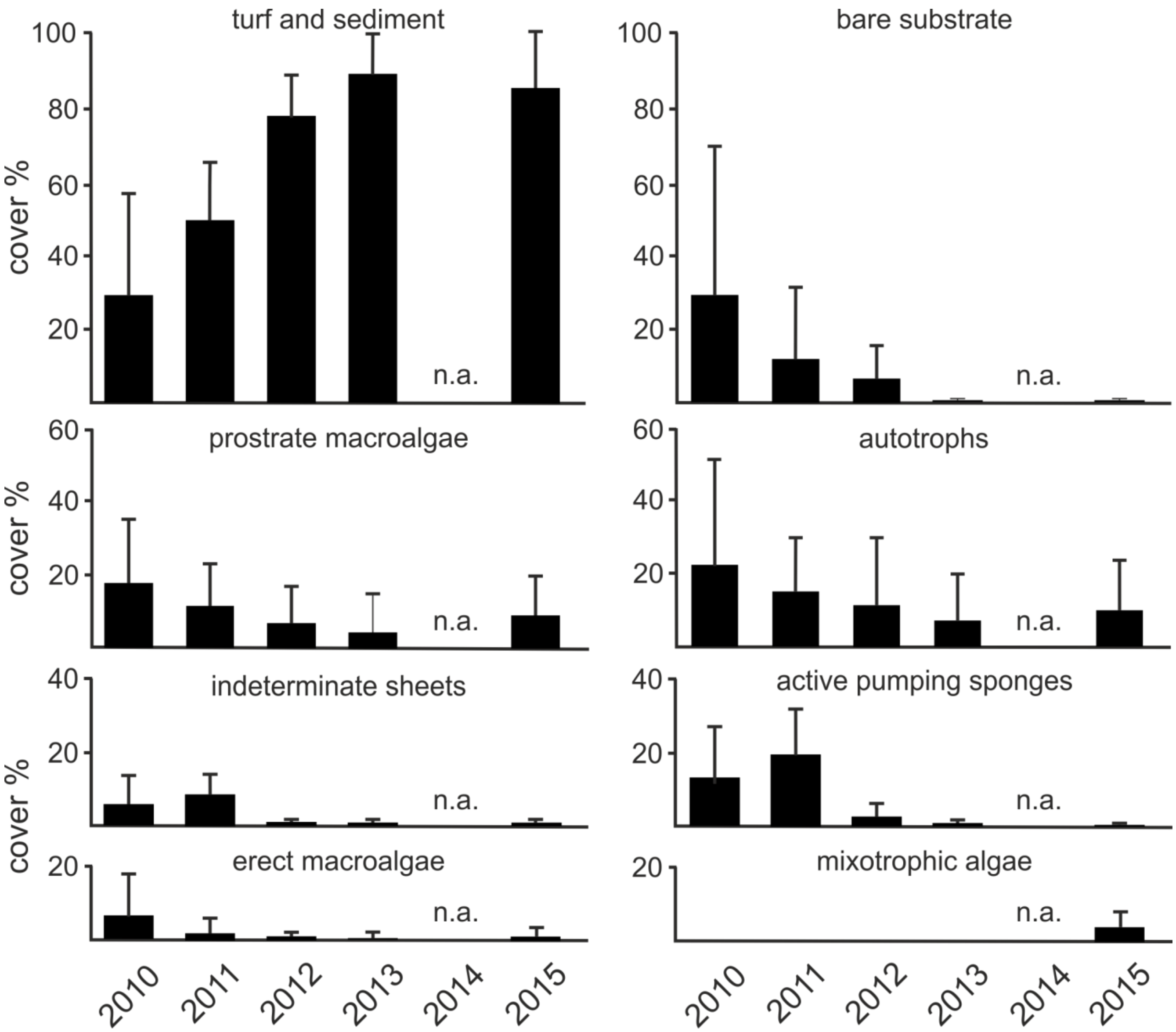


Figure 3

Figure 2



turf and sediment
bare substrate
prostrate macroalgae
autotrophs
indeterminate sheets
active pumping sponges
erect macroalgae
mixotrophic algae
cover %
100
80
60
40
20
n.a.
2010
2011
2012
2013
2014
2015

Figure 4

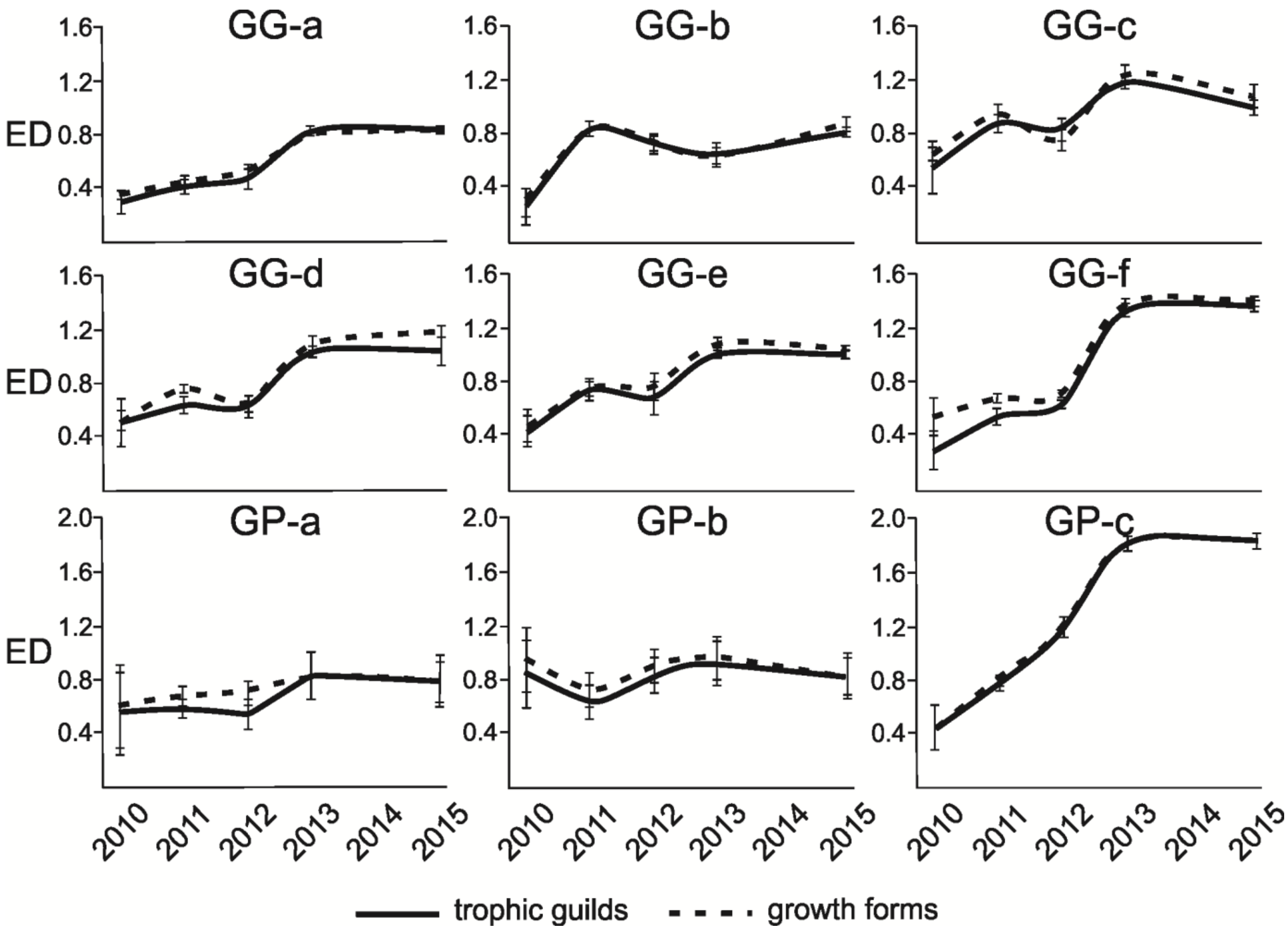

GG-a
GG-b
GG-c
GG-d
GG-e
GG-f
GP-a
GP-b
GP-c
ED
0.4
0.8
1.2
1.6
2.0
2010
2011
2012
2013
2014
2015
trophic guilds
growth forms

Figure 5

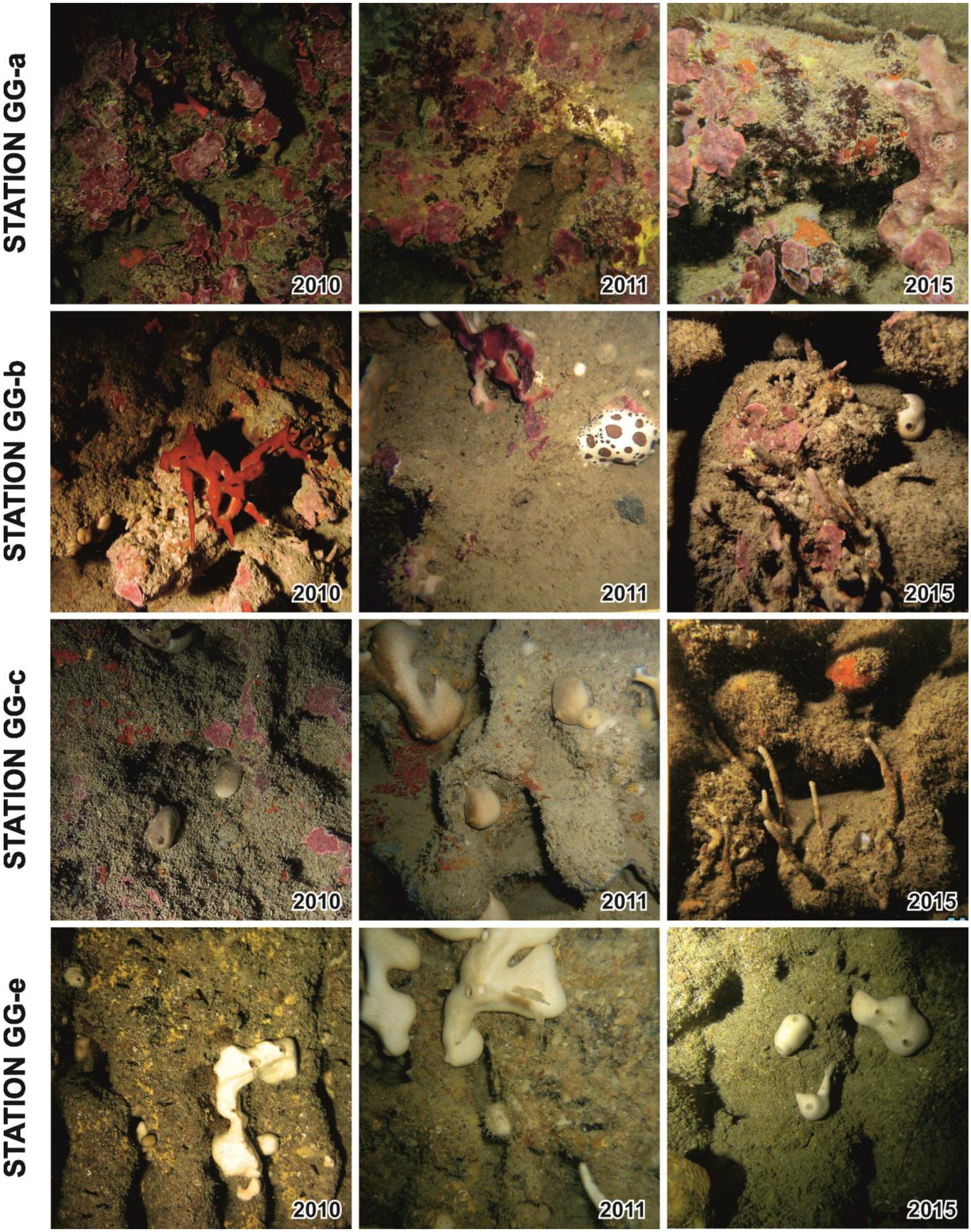

STATION GG-a
2010
2011
2015
STATION GG-b
2010
2011
2015
STATION GG-c
2010
2011
2015
STATION GG-e
2010
2011
2015

Figure 6

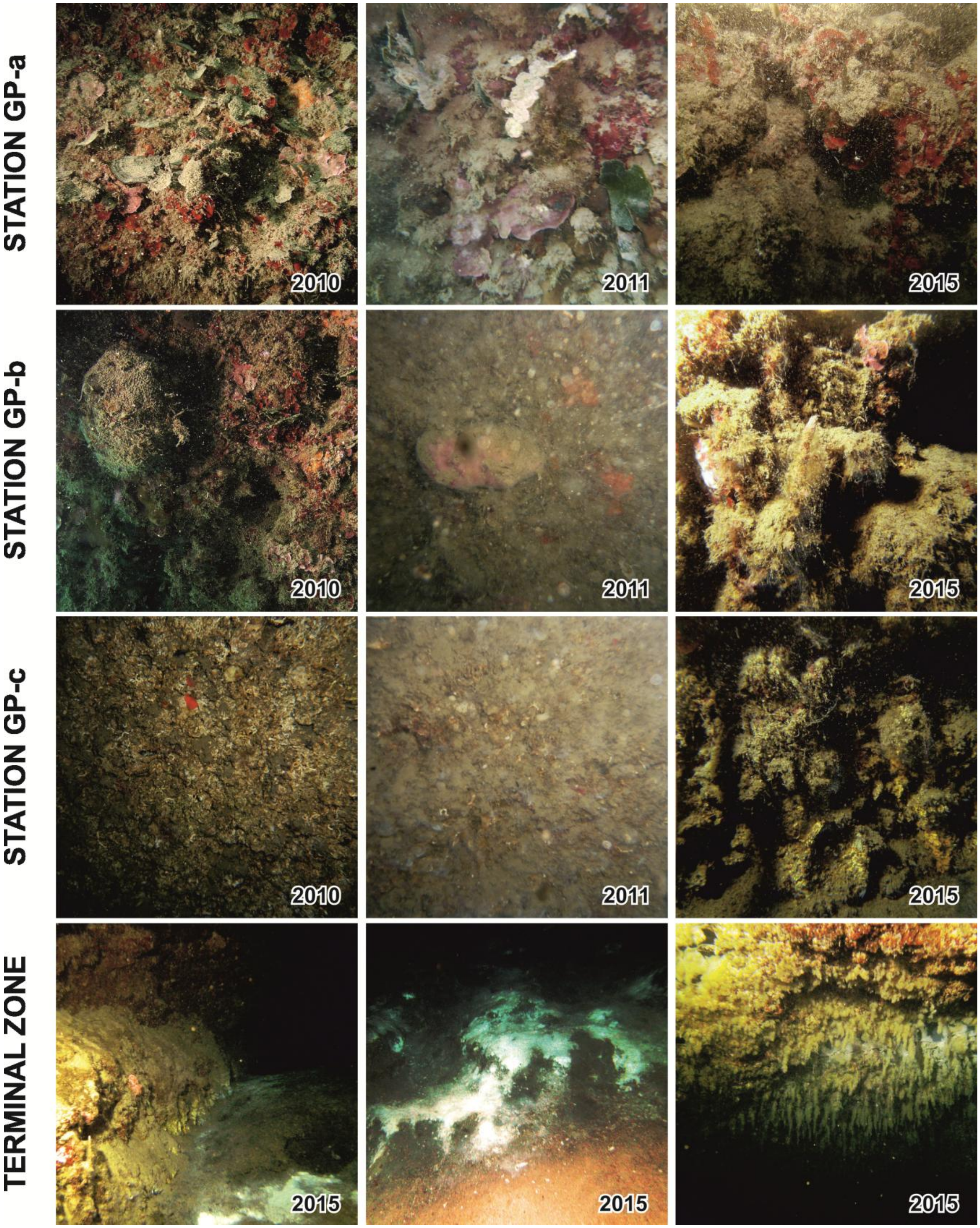

STATION GP-a
2010
2011
2015
STATION GP-b
2010
2011
2015
STATION GP-c
2010
2011
2015
TERMINAL ZONE
2015
2015
2015

Figure 7