The influence of light attenuation on the biogeomorphology of a marine karst cave: a case study of Puerto Princesa Underground River, Palawan, the Philippines.

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Abstract

Karst caves are unique biogeomorphological systems. Cave walls offer habitat for microorganisms which in-turn have a geomorphological role via their involvement in rock weathering, erosion and mineralisation. The attenuation of light with distance into caves is known to affect ecology, but the implications of this for biogeomorphological processes and forms have seldom been examined. Here we describe a semi-quantitative microscopy study comparing the extent, structure, and thickness of biocover and depth of endolithic penetration for the Puerto Princesa Underground River system in Palawan, the Philippines, which is a natural UNESCO World Heritage Site.

Organic growth at the entrance of the cave was abundant (100% occurrence) and complex, dominated by phototrophic organisms (green microalgae, diatoms, cyanobacteria, mosses, and lichens). Thickness of this layer was $0.28 \pm 0.18 \text{ mm}$ with active endolith penetration into the limestone (mean depth = $0.13 \pm 0.03 \text{ mm}$). In contrast, phototrophs were rare 50 m into the cave and biofilm cover was significantly thinner ($0.01 \pm 0.01 \text{ mm}$, $p < 0.000$) and spatially patchy (33% occurrence). Endolithic penetration here
was also shallower (< 0.01 mm, \( p < 0.000 \)) and non-uniform. Biofilm was found 250 m into
the cave, but with a complete absence of phototrophs and no evidence of endolithic
bi ero sion.

We attribute these findings to light-induced stress gradients, showing that the influence of
light on phototroph abundance has knock-on consequences for the development of
limestone morphological features. In marine caves this includes notches, which were most
well-developed at the sheltered cave entrance of our study site, and for which variability in
formation rates between locations are currently poorly understood.

**Key-words:** biogeomorphology; marine cave; microbiology; bioerosion; karst; Palawan.

### 1. Introduction

Caves provide a unique habitat for rock-dwelling microorganisms that in turn are thought to
have a geomorphological role via their involvement in weathering, erosion and
mineralisation (Barton, 2006; Barton and Jurado, 2007; Riquelme and Northup, 2013).
Cave characteristics such as dimension, morphology, location, orientation and lithology
have an important influence on the structure of the biological communities found in these
environments (Lamprinou et al., 2012). This appears to be especially important for
limestone caves, where biogeomorphological interactions between rock and biota are
strongest (e.g., Cañveras et al., 2001; Jones, 2010; Pasic et al., 2010).

The ecology of cave walls (including marine caves) is characterised by a decrease in
biomass towards the interior, resulting from the presence of marked environmental
gradients, including light, oxygen, temperature and nutrient availability. Light attenuation is
particularly critical for phototrophs (Gili et al., 1986). In terrestrial caves in Spain, for
example, Roldán and Hernandez-Mariné (2009) found that biofilms comprised of
cyanobacteria, green microalgae, diatoms, mosses and lichens on the walls and floors were thinner farther from the entrance, and had lower species diversity. Similarly, Cuezva et al. (2009) found that microbial colonies on rock surfaces are concentrated at the entrance of Altamira Cave, Spain, and that these microorganisms are involved in biomineralisation and CaCO₃ deposition. While the influence of light attenuation on cave microbial communities is clear, there has been very little attempt to relate these patterns to biogeomorphological processes. This is an important research gap because the relative contribution of biological processes (i.e., bioweathering, bioerosion, and bioprotection) to the formation of distinct morphological features associated with cave systems is relatively poorly understood. For marine caves this includes notches, which are very common and well developed on Mediterranean and tropical limestone coasts (e.g., Trudgill, 1976; De Waele et al., 2009; Furlani et al., 2011; Moses, 2012; Pirazzoli and Evelpidou, 2013). The mechanisms involved in notch formation have been debated (Furlani et al., 2011), but probably involve a combination of chemical, biochemical, biomechanical and physical processes (i.e., waves) (see De Waele and Furlani, 2013, and references therein). Some researchers suggest that bioerosion is the key parameter controlling the rate of marine notch formation (e.g., Evelpidou et al., 2012; Pirazzoli and Evelpidou, 2013; Boulton and Stewart, in press), yet direct quantitative evidence of bioerosion is scarce (but see Furlani and Cucchi, 2013).

Here we describe a study aimed at addressing this knowledge gap using the Puerto Princesa Underground River cave system as a case study. The study had three main aims: (1) to examine the presence and characteristics of ecology (focusing on microorganisms) on cave walls in relation to distance from the entrance and therefore availability of light; (2) to determine the biogeomorphological significance of these organisms in a context of bioweathering and bioerosion, and thereby; (3) contribute to
understanding of biogeomorphological processes in the development of marine cave morphological features, including notches.

2. Study Site

Palawan is located between 11°50' and 12°20' latitude north, and 117°00' and 120°20' longitude east, in the south-western part of the Philippines archipelago. Climate is characterised by a dry season (November to May) and a wet season (June to October), with stable temperatures throughout the year ranging from about 26°C to 28°C (Piccini and Iandelli, 2011). The island is narrow and elongated, mostly mountainous, and divided into three geologic sectors by two north-south oriented tectonic depressions. The Saint Paul Dome karst ridge divides the northern from the central sector, located east of Ulugan Bay about 50 km northeast of Puerto Princesa (Figure 1). The ridge covers an area of about 35 km² (10 km long and roughly 4 km wide) and is formed of massive to roughly bedded (400 m thick) light to dark grey Oligocene-Miocene limestone rich with fossils (Hashimoto, 1973). The site is a National Park and an UNESCO natural World Heritage Site.

Structurally the area consists of a multiple northwest dipping homoclinal relief, limited by northeast-southwest oriented faults that control the general morphology of the karst landscape, including the orientation of dolines and the development of major caves. One such cave system is the Puerto Princesa Underground River (PPUR) complex, a 32 km long cave that consists of an active branch developing at the present sea-level, and two relict levels of huge tunnels and large breakdown chambers at approximately 5-10 and 60-80 m above present sea-level. The cave has had a long and multiphase evolution coupled with uplift phases and sea level fluctuations. Several morphological features suggest that the lowest and presently active level of the cave is inherited (e.g., Blanco Chao et al., 2003), probably formed during the Middle-Late Pleistocene (Piccini and
Iandelli, 2011). These features include notches at +12.4 m above mean sea level, and large and corroded speleothem masses that are interbedded with alluvial deposits. Features that indicate former water levels are present up to 5–6 km upstream from the coastal spring, where a notch is evident at +7–8 m that is correlated to a notch on the coastal cliff about +7 m above present sea level (Maeda et al., 2004; Omura et al., 2004). These notches date back to the MIS 5e interglacial phase (about 125,000 years BP) (Linsley, 1996).

At the present sea-level a combination of physical erosion, bioweathering, bioerosion and chemical dissolution are thought to have contributed to the development of a deeply-carved notch (up to 2 to 3 m) along the coastline (Figure 2a). The notch is present in areas both exposed to the direct action of waves and more sheltered alcoves, which in itself indicates that processes other than wave action are important for formation. In the entrance zone of the cave, where wave energy is greatly reduced, the notch is about 0.8-1.2 m deep (Figure 2b). In the inner part of the cave the present-day notches are rarely deeper than 0.4-0.6 m, except where freshwater tributaries occur.

3. Materials and Methods

Samples of limestone rock from the PPUR were collected for examination to compare biological and biogeomorphological features. Drill core samples (1 cm diameter x 2 cm deep) and chippings of similar dimensions were obtained by boat from the cave walls at three distances from the entrance (SP0 = cave entrance with ‘full light’, SP1 = 50 m from the entrance with ‘semi-light’, SP2 = 250 m from the entrance in ‘darkness’) and at three different heights from the mean water level (suffix 1 = 20–30 cm above mean waterline, 2 = 40–50 cm above mean waterline, 3 = 70–80 cm above the mean waterline) (Table 1, Figure 2c–e). All samples were therefore taken from within the tidal zone, where organic activity in the form of biofilms and entrusting organisms was visually abundant in
comparison to areas above high water. Although grazing organisms (including limpets and chitons) may contribute to limestone bioerosion, these were rare in this study location and never abundant.

3.1. Microscopic analyses

Light microscopy and scanning electron microscopy (SEM) were used to analyse the samples. A light microscope (Leica MZ 10 F) was first used to characterise and compare the nature (abundance, structure, types and diversity) of biological cover on the top surface of core samples taken from the three distances from the entrance (0 m, 50 m and 250 m) and the three heights above the mean waterline (20–30 cm, 40–50 cm, 70–80 cm). In total 43 cores were examined, 15 from both the entrance at 50 m depth, and 13 at 250 m depth. Each core was observed with artificial lighting from above (using an optic fiber) at increasingly higher magnification and photographs were taken using DeltaPix Insight computer software.

For comparison between samples, the organisms forming the organic layer (where present) were broadly classified into the following morphological groups: green microalgae, filamentous cyanobacteria, coccoid cyanobacteria, diatoms, mosses, lichens, actinobacteria and invertebrates. The entire surface of each core was examined and the presence/absence of each group was recorded in each case. Presence/absence of morphological groups was compared as a percent occurrence (e.g., Taylor and Viles, 2000) for each set of cores from each distance/height combination (Table 1).

The nature (extent, structure and thickness/depth) of interactions between surface microorganisms and the limestone substratum was further examined using SEM. As the light microscopy revealed consistent patterns in microbial cover for all tide levels (see Section 4 and Section 5) SEM observations were made for samples from one height (40–50 cm above mean water) at each of the distances into the cave (i.e., SP0/2, SP1/2 and
SP2/2). For these observations core samples were fractured using a hammer and chisel. This was done from the side, with cores placed on a piece of foam so as to minimize disruption of the surface of interest. Prior to SEM observation the air-dry samples were mounted on aluminium stubs using carbon cement, with the upper colonized surface to the perpendicular (i.e., a cross-section view of the rock–biota interface) (e.g., Viles, 1987; Viles et al., 2000). Samples where then gold sputter coated and observed using a Cambridge Sterioscan 90 SEM.

To characterise and measure the rock–biota interface, the top edges of three randomly selected and fractured cores from each distance into the cave were observed. Observations were first made at low magnification (x50) and then at progressively higher magnifications until distinction could be made between any modified zones (typically x200–500). For each core sample five micrographs were taken with roughly equal spacing along their top edge (i.e., 3 full turns of the SEM navigation wheel at x50, equivalent to around 1 mm spacing). Micrographs were then scaled using ImageJ computer software to enable semi-quantitative measurements of: (a) surface organic cover thickness, and (b) depth of organism (endolithic) penetration, where present, measured parallel to the rock surface (e.g., Coombes et al., 2011).

4. Results

4.1. Biological characterisation: light microscopy

Using the classification of microorganisms adopted here, we found little variation in occurrence in relation to height above the waterline. Therefore, data from the three heights were collapsed into one group per distance from the cave entrance. For these three groups (0 m, 50 m and 250 m), microbiological communities varied significantly as indicated by a visible colour change of the rock surface (e.g., Figure 2c–e); cores from the cave entrance were visibly greener, those 50 m from the entrance were green-grey, and
those 250 m from the entrance were dark brown. Microscope observations showed that these colour differences are attributed to marked variations in the presence/absence of phototrophs (Figure 3).

As expected, biofilm at the cave entrance was dominated by a range of phototrophic organisms (filamentous and coccoid cyanobacteria, green microalgae, diatoms, mosses, and lichens) forming a photosynthetic layer (Figure 3). The structure of this organic layer was complex and stratified (Figure 4a–c). Biofilm was also observed on samples from farther within the cave, but this was simple in structure and spatially-patchy in comparison to those at the cave entrance (Figure 4d–f). Importantly, phototrophs were rare at 50 m and completely absent 250 m depths (Figure 3). Supplementary qualitative SEM observations of the top surfaces of cores confirmed that there were markedly more biological cells (especially filamentous algae and cyanobacteria) on samples taken from the cave entrance (e.g., Figure 5a). Microbial cover was notably less clear for 50 m and 250 m samples, although there were some crusting forms of possible biochemical origin (e.g., Figure 5b–c) similar to those observed to develop on limestone exposed to intertidal conditions in the UK (Coombes et al., 2011).

In addition to microorganisms, some invertebrate species were found including those from the phyla Mollusca and Arthropoda (Figure 3, Figure 6). In contrast to phototrophic microorganisms, which showed little variation in relation to height, invertebrates were only found on samples originating closest to the waterline (20–30 cm), irrespective of distance from the entrance.

4.2. Rock-biota interactions: SEM

Figure 7 shows characteristic cross-section views for fractured rock cores taken at the three distances from the cave entrance. Figure 8 shows measurements of biocover thickness on top of the rock surface, and depth of active penetration of microorganisms
into the substrate. At the cave entrance, a complex and complete biocover exists (100% occurrence) of biological filaments and single-celled algae and diatoms (Figure 7a), corresponding well to top-surface observations. There was evidence that some of the filaments within this layer were heavily mineralised. The thickness of the epilithic layer averaged 0.28 ± 0.18 mm (Figure 8), although this was spatially variable as indicated by a relatively high standard deviation. It was noted that biofilm thickness was typically greatest in association with surface irregularities/depressions on the rock. Observation at higher magnifications (x300+) showed that microorganisms are actively penetrating the limestone at the cave entrance, where in situ organic filaments were visible in a relatively uniform zone of microbial boring (Figure 7a). This bioeroded zone had an average depth of 0.13 ± 0.03 mm (Figure 8).

In comparison, the thickness of biocover 50 m into the cave was significantly thinner than at the entrance (0.01 ± 0.01 mm, Student’s t(14) = 5.71, p < 0.000, Figure 8) and spatially patchy (present in only 33% of observations at this location). There was some evidence of bioerosion of these samples but this was less distinct and markedly less uniform (e.g., Figure 7b). The depth of this altered zone was significantly shallower than at the entrance (a mean depth of 0.01 ± 0.01 mm, Student’s t(16) = 13.46, p < 0.000, Figure 8), and may be attributed to chemical weathering (evidenced by disaggregation and increased pore space, Figure 7b) alongside any biogeomorphological alteration. No surface biocover of measureable thickness was observed for samples taken from 250 m into the cave (Figure 8). Equally, there was no evidence of endolithic organisms in the 250 m samples, although there was some morphological evidence of alteration of the very top surface, possibly via chemical means (Figure 7c).

5. Discussion

5.1. Height above the waterline
Height above the waterline did not have a significant influence on the composition and structure of phototrophic biofilms; at this scale of observation, few differences were found between respective groups of samples from the three heights. The tidal range in this cave is 50–120 cm, suggesting that the influence of rising and falling tides may preclude any observable difference in microbiological communities associated with tidal height for our samples. This can be explained by the fact that microorganisms forming intertidal biofilms are relatively tolerant to desiccation given that the matrix of extracellular polymeric substances in which they are embedded can retain moisture (Decho, 2000). Furthermore, the high moisture content of the air near the entrance and farther into the cave (almost 100%) limits evaporation and maintains the walls wet during the low tide. Further investigations to compare microbial communities occurring above and below the tide line would help clarify whether the kinds of biogeomorphological processes observed are contingent on tides, especially at the entrance where water and desiccation stress (rather than light) are likely to limit growth. In contrast to microbial communities, height was found to be an important for the presence of motile marine invertebrates, which were only found on samples taken closest to the waterline (20–30 cm). This indicates that these organisms are more sensitive to variations in moisture (and desiccation) resulting from tidal fluctuations (e.g., Gosselin and Qian, 1997).

5.2. Distance from the cave entrance

Overall, there was a significant decrease in taxa richness and biomass with distance into the cave system. At the cave entrance, a complete cover of biofilm composed of several phototrophic groups was present, with a well-stratified structure and a maximum thickness of 0.49 mm. Barton (2006) suggests that the two most common bacterial forms in cave communities are bacilli (in chains, as streptococci) and cocci (sometimes in pairs, as diplococci), which agrees well with our observations. Some encrusting and epilithic
organisms were also present 50 m into the cave, but the biofilm here was significantly
thinner and there were few phototrophic forms. Deeper into the cave (250 m from the
entrance) biofilm cover was patchy and poorly stratified, with no phototrophic organisms.
These observations conform to previous studies that have observed a reduction in the
presence of microalgae and cyanobacteria species in association with light attenuation in
cave systems (Roldán et al., 2004; Roldán and Hernandez-Mariné, 2009). Notably,
phototrophic microflora are restricted to the vicinity of the cave entrance (e.g., Pantazidou,
1996; Albertano and Urzi, 1999; Mulec, 2008). Light intensity also appears to influence the
ratio of different organism groups (e.g., algae to bacteria, Figure 3), probably in
combination with variations in other environmental factors such as the availability of
nutrients and organic material, and the efficiency of gaseous exchange (Ohki and Gantt,
1983). The availability of light is nevertheless the critical limiting factor for autotroph
occurrence and abundance, even where other environmental conditions may be
favourable.

5.3. The biogeomorphological significance of light attenuation in caves
SEM cross-section observations showed greatest evidence of organic modification of the
limestone substratum (i.e., bioerosion) at the cave entrance, corresponding to the location
where lithic organisms were most abundant. Here, a bored zone averaging 0.13 mm deep
was observed below the upper phototrophic biofilm layer. In this bored zone, mineral rock
and organic structures were closely associated, with filaments visibly penetrating into the
rock alongside boreholes and tunnelling artefacts (e.g., Viles et al., 2000; Naylor and Viles,
2002; Coombes et al., 2011). There was also evidence of mineralisation of calcite in
association with organic structures, which has been noted as an important modifying
process in karst caves (e.g., Cuezva et al., 2009). The extent to which such processes
represent possible bioprotective mechanisms in marine caves requires much more
investigation. On the other hand, organic breakdown of the rock (i.e., microbial penetration into the surface) was particularly apparent in association with topographic depressions, where the surface biocover was also usually thickest. This indicates that bioerosion is at least partly responsible for creating (or enhancing) the marked mm–cm scale physical complexity of the rock surface at the cave entrance compared to smoother, fluted rock in the interior part of the cave (Figure 2c–e). A positive feedback mechanism likely exists here, whereby topographic depressions created by bioerosion offer favourable microsites (i.e., wetter and cooler) for subsequent microbial growth, but such causal relationships are difficult to corroborate.

Phototrophic organisms, particularly cyanobacteria, are known to be effective bioeroders of limestone rock, and although the precise mechanisms involved are debated, this probably occurs via chemical means including acidulation during photosynthesis (Garcia-Pichel, 2006). Beyond the cave entrance, light limits the presence of these phototrophs, and consequently the relative importance of bioerosion by endolithic microbes is markedly reduced. This transition between bioerosive biofilms dominated by phototrophs and epilithic, non-photosynthetic biofilms may be relatively abrupt; our sampling indicates that a distance of 50 m is sufficient to give rise to a significant reduction in phototroph abundance and associated bioerosion, although this will likely vary depending on cave entrance morphology and aspect. Other studies have similarly found that microorganisms in the transition zone between well-illuminated parts of caves (i.e., at the entrance) and areas of darkness (the ‘twilight zone’) only occupy the epilithic niche (e.g., Jones, 1993) and as such are not involved in rock boring. Microbes deeper in caves may also contribute to rock breakdown via other means (such as biochemical etching), but this could not be easily distinguished based on these microscope observations. Our findings do clearly show, however, that the extent (and probable rate) of rock breakdown and topographic development is greatest at the cave
entrance, where phototrophic organisms are able to dominate. In contrast, the relative
geomorphological significance of endolithic microorganisms is markedly reduced in the
cave interior. Here, chemical processes such as mixing corrosion are likely to be more
important than biogeomorphological process. For example, in their broad-scale survey of
submerged notches along the Adriatic coast, Furlani et al. (2014) conclude that freshwater
mixing (from groundwater and fluvial inputs) is a major factor in their development
alongside bioerosive processes. The relative importance of notch formative processes is
significant given that notches are commonly used as indicators of relative sea-level
change, particularly in carbonate rocks (e.g., Pirazzoli and Evelpidou, 2013; Boulton and
Stewart, in press) and in tectonically active areas where changes may be gradual and/or
abrupt (Evelpidou et al., 2012). Our results demonstrate that, where present, phototrophs
are probably significant in their contribution to notch formation in this area, especially in the
controlled setting of cave entrances where the direct influence of physical wave action is
markedly reduced.

6. Conclusions

This study demonstrates how the known influence of light attenuation on the microbial
ecology of cave systems has important, but largely unstudied, biogeomorphological
consequences. This occurs via the maintenance of a gradient of lithic niche occupation,
from biofilms dominated by bioerosive endolithic phototrophs at cave entrances (alongside
biomineralising epiliths) to non-photosynthetic epilithic biofilms in cave interiors. The
transition between these types of growths was in the order of 50 m for the Puerto Princesa
Underground River cave complex. Further work is now required to determine the
consistency of this spatial transition between different locations.

Based on these observations we conclude that bioerosion of these cave walls (in the tidal
zone) is spatially controlled by light availability; bioerosion was only active at the current
cave entrance where there is sufficient light for photosynthetic microorganisms to exist, and as such it is here that microbes are most directly involved in the morphological development of the limestone surface. Whilst bioerosion was found to be negligible beyond 50 m from the cave entrance, other biologically-mediated processes may be operating. These probably include biocorrosion and possibly bioprotection via mineralisation, but this requires further investigation particularly in a marine context. More broadly, our observations have demonstrated the utility of geomorphological process studies for elucidating controls on the morphological development of marine dissolutional cave systems. This includes the processes contributing to the formation of marine notches, and how the relative contribution of biological, chemical and physical weathering is complicated by spatial variations in the abundance and niche occupation of microbial biofilms. Our observations further suggest that relationships between notch lateral depth and distance from the entrance of caves probably exist as a function of relative phototroph abundance. This is certainly appears the case for the Puerto Princesa Underground River cave, where bioerosion artefacts are present to significantly greater depths in the rock at the entrance. This research demonstrates that current process biogeomorphological studies can aid understanding of marine notch formation rates and mechanisms, which is critical for employing notches in sea-level reconstruction (e.g., Furlani and Cucchi, 2013; Pirazzoli and Evelpidou, 2013).

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References


Furlani, S., Cucchi, F., 2013. Downwearing rates of vertical limestone surfaces in the intertidal zone (Gulf of Trieste, Italy). Marine Geology, 343, 92-98.


**Table 1.** Rock sampling in relation to distance into the Puerto Princesa Underground River system and height above mean water level.

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Figure Captions:

Figure 1. Location map of the Saint Paul karst area. Location of the Puerto Princesa Underground River system (PPSE) indicated.

Figure 2. Photographs showing: (a) well-developed notches of the Saint Paul karst area; (b) notch development at the cave entrance; (c) core sampling at the cave entrance; (d) core sampling 50 m into the cave, and; (e) core sampling 250 m into the cave (scale bars indicate 10 cm).

Figure 3. Occurrence (%) of phototrophic groups of microorganisms and invertebrates in light microscope observations of samples taken at three distances from the cave entrance (0 m n = 15, 50 m n = 15, 250 m, n = 13).

Figure 4. Varying structure of biofilm on the surface of samples from different locations in the cave: (a) thick and stratified biofilm at the cave entrance; (b and c) complex biofilm with visible filamentous algae at the cave entrance; (d) simple epilithic biofilm characteristic of surfaces 50 m from the entrance, and; (e and f) 250 m from the cave entrance (scale bars = 1 mm).

Figure 5. SEM micrographs of top surfaces of samples taken at: (a) 0 m, (b) 50 m, and (c) 250 m from the cave entrance (all samples are 40–50 cm above the mean waterline, magnification and scale as shown).

Figure 6. Invertebrates present on samples sampled 20–30 cm from the mean waterline: (a) Mollusca (bivalves) at the cave entrance; (b) Mollusca (gastropods) at the cave entrance; (c) Mollusca (bivalves) 250 m from the cave entrance; (d) Arthropod crustacean at the cave entrance (bars = 1 mm).

Figure 7. SEM observations of surfaces in cross-section for samples taken at: (a) 0 m from the entrance ([i] thickness of biocover, [ii] zone of bioerosion, [iii] close-up view of in situ filaments penetrating the rock); (b) 50 m from the entrance ([i] possible zone of biochemical alteration); (c) 250 m from the cave entrance ([i] amorphous structures, of
possible chemical rather than organic origin) (all samples were from 40–50 cm above the mean waterline, magnification and scale as shown).

**Figure 8.** Indicative thickness of biocover (mean ± SD) and depth of active penetration into the substratum (mean ± SD) by microorganisms at different distances within the cave (all samples from 40–50 cm above mean waterline, n = 15).


Furlani, S., Cucchi, F., 2013. Downwearing rates of vertical limestone surfaces in the intertidal zone (Gulf of Trieste, Italy). Marine Geology, 343(0), 92-98.


