



Seasonal dynamics of *Hildenbrandia rubra* in a Mediterranean marine cave: insights into the algal community and biodiversity

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Abstract

Marine caves, often overlooked hotspots of biodiversity, provide unique habitats for specialized species. Located on the Mediterranean coast, Rosh HaNiqra is a midlittoral cave renowned for its vibrant epilithic algal community. In the study reported here, we explored the light environment, algal composition, and ecological dynamics of the Rosh HaNiqra cave. Light measurements revealed that illumination in the cave ranged from 0.5 to 2.5% of the sunlight recorded at the mouth of the cave, with variations across seasons and times of day, peaking during summer, and an increase in red wavelengths towards sunset. Biodiversity assessments, including *rbcL* gene sequencing studies, identified *Hildenbrandia rubra* (red alga) as the principal alga, together with *Blidingia dawsonii* (green alga), cyanobacteria, and mosses, forming a “Balcony of Colors”. The cave's conditions were found to be most favorable for *H. rubra* in the autumn through early winter, when the cave provides a critical refuge where this alga thrives in stable, low-light conditions, demonstrating high photosynthetic efficiency even in reduced light. Our study of algal distribution showed seasonal fluctuations, with peak coverage during warmer months and a decline in winter. This study not only deepens our understanding of Rosh HaNiqra's cave ecosystem but also provides an essential baseline for future ecological and conservation research in marine caves.

Keywords Rosh HaNiqra · Mid-littoral caves · Epilithic algae · Rhodophyta · *Hildenbrandia*

Introduction

The littoral fringe serves as a transitional zone between land and sea within the littoral area of marine ecosystems. According to the European Union Habitats Directive, marine ecosystems encompass six distinct habitats: supralittoral rock, midlittoral rock, lower midlittoral rock, infralittoral rock, circa-littoral rock, and bathyal and abyssal rock caves (European Commission 2007). Coastal caves are classified based on their origin and position, ranging from below sea level to above sea level. Submarine caves are fully submerged, with an entrance located below sea level, while seaside or coastal caves can be either submerged or partially exposed. Specifically, midlittoral caves are situated within the intertidal zone, with their entrance at sea level.

Wave action is a primary force in the development of midlittoral caves. These formations are prevalent on rocky coasts, particularly along fault lines where sea cliffs exhibit zones of weakness. This geological setting facilitates the accelerated removal of rock and the creation of caves at sea level (Gerovasileiou and Voultsiadou 2012). The elevation

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or subsidence of coastal platforms near fault lines can significantly affect relative sea levels. Consequently, midlittoral caves often display evidence of erosion at 1 m above and below the wave base, which reflects past sea levels (Morhange et al. 2006; Marriner and Morhange 2007). Grottos, cave-like formations that can be naturally occurring or man-made, are commonly found in coastal settings such as cliffs, in hillsides, or near bodies of water. These spaces, which served various functions in prehistoric and historical periods, are also affected by tidal fluctuations (Waterstrat et al. 2010).

The Rosh HaNiqra cave, the sole midlittoral cave in Israel, is notable for its unique geological and ecological features. Stretching over 3 m in length, this cave is part of a complex network of 200-m-long cavernous tunnels formed by sea action on calcareous rock. The seismic activity in the late Holocene of the Eastern Mediterranean led to the continuous rise of the Western Galilee coastline, with regions of 120- to 140-cm-elevated abrasion platforms from Turkey to Lebanon and Rosh HaNiqra (Pirazzoli 2005; Morhange et al. 2006; Marriner and Morhange 2007). In Rosh HaNiqra, sea level rise has resulted in a sediment cover at the cave's entrance, with 2 m of sediment obscuring the 3- to 5-m-deep opening (Sela 1973).

Midlittoral caves formed along faults are characterized by canyon-like or angled passages, which can influence light distribution within the cave. One such example is the Seal Canyon Cave on Santa Cruz Island, which is located off the southwestern coast of Ventura, California in USA, where sunlight remains visible even from the back of the cave at 189 m from the entrance (Bunnell and Kovarik 2013). Similarly, in the "canyon-like" midlittoral cave of Rosh HaNiqra, direct sunlight penetrates as far as 200 m into the cave during the summer months. The intensity and duration of this sunlight vary throughout the year, influenced by cave morphology, proximity to the equator, and seasonal changes (Secord and Muller-Parker 2005; Knittweis et al. 2015). Light penetration in midlittoral caves generally follows an exponential decay pattern, with photosynthetically active radiation (PAR) declining to about 10% of ambient light halfway into the cave and to approximately 1% at the rear (Secord and Muller-Parker 2005).

Few researchers have focused on midlittoral caves due to their challenging and often inaccessible environments. These caves present extreme abiotic conditions, including low light, frequent desiccation, variable temperatures, and occasional direct light (Giakoumi et al. 2013). Despite the Rosh HaNiqra cave being the only midlittoral cave in Israel that is > 3 m in length, no comprehensive taxonomic study on the algal communities has been published. Previous reviews of taxonomic studies in Israel noted only two reports on species of the marine alga genus *Hildenbrandia*, specifically in the infralittoral zone of Mikhmoret in the winter

of 1974 (Lundberg 1996). At Rosh HaNiqra, research has predominantly focused on the infralittoral zone, leaving the grottos understudied (Einav and Israel 2008). The deepest part of midlittoral caves typically harbors red algae (division Rhodophyta), while brown algae (class Phaeophyceae) and green algae (division Chlorophyta) are more commonly found closer to the cave's entrance (Marti et al. 2004; Knittweis et al. 2015).

The genus *Hildenbrandia* (Hildenbrandiales, Rhodophyta) comprises 11 species, of which ten species are marine algae. *Hildenbrandia rubra* (Sommerfelt) Meneghini 1841 (Guiry and Guiry 2022), a non-calcareous perennial crustose rhodophyte, occurs in estuarine environments, on harbor structures, rocky substrates, and cave surfaces. This species exhibits a vertical distribution from upper littoral caves to sublittoral depths of at least 12 m and represents a key taxon in Mediterranean midlittoral cave communities (Ramos-Esplá et al. 2015). There are documented records of *H. rubra* in the infralittoral zones of Barbra and Lebanon, as well as in the midlittoral caves of Beirut (Lakkis and Novel-Lakkis 2000; Boudouresque 1971; Lobban and Harrison 1994; Kaehler and Williams 1996; Kanaan et al. 2014). Although *H. rubra* demonstrates widespread distribution in mid- and infralittoral zones, its presence in midlittoral caves is attributed to extreme environmental conditions and reduced interspecific competition (Connor et al. 2003a, b; Mayer et al. 2016; Tyler-Walters 2016). However, *H. rubra* shows notable physiological plasticity, tolerating variable light intensities, temperatures ranging from -17°C to 27°C , and fluctuating hydration conditions (Lobban and Harrison 1994; Kim and Garbary 2006).

This study represents a pioneering survey of the Rosh HaNiqra cave and its epilithic algal flora. Established as a marine reserve in 1972 and expanded to a 15-km-wide marine reserve in 2020, Rosh HaNiqra attracts over 300,000 visitors annually, leading to intermediate levels of tourism-induced disturbance. Documentation of the epilithic algal community (EPC) within this cave system is essential for its conservation and management. The aim of this study was to identify the presence of the red macroalga *Hildenbrandia* and evaluate changes in model accuracy and performance for habitats with varying distributional patterns by employing both classical taxonomic methods and modern analytical techniques.

Materials and methods

Light measurements

Light measurements were recorded in calm sea conditions in two stations of the Rosh HaNiqra grotto (Fig. 1). Light spectrum and intensity were recorded directly outside of the

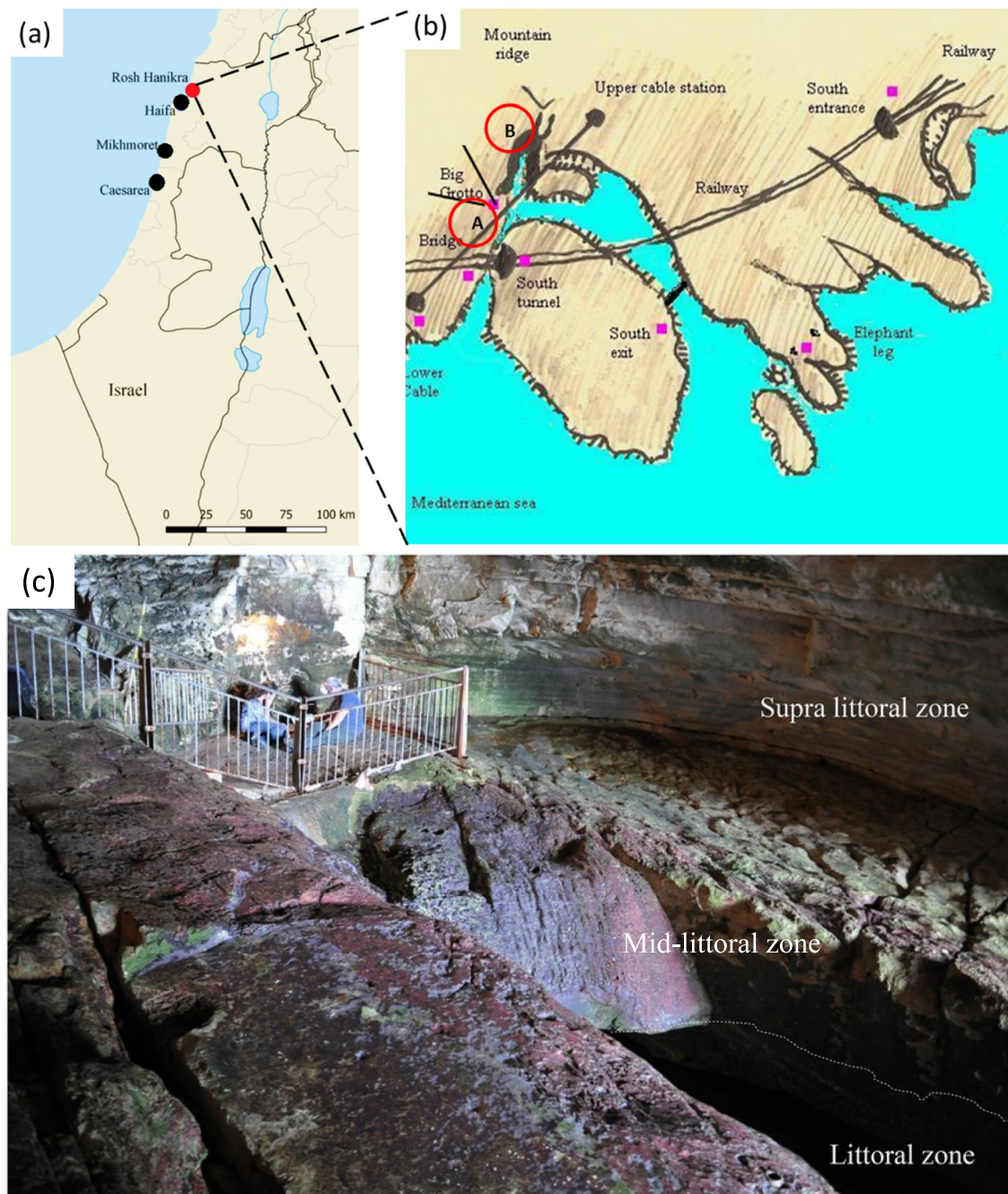


Fig. 1 The Rosh HaNiqla grottos located in Haifa, Israel ($33^{\circ}05'2''$ N, $35^{\circ}06'16''$ E) (a), showing the study stations A and B (b), which are located in the main grotto (adapted from Sela et al. 1973 and revised) and inside the Rosh HaNiqla midlittoral cave (c)

cave opening (station A) and at the maximum extent of the cave (station B) (Fig. 1). The inside station (B) was located on the balcony present within the cave, 200 m away from the cave opening and towards the inside, and 3 m above average sea level; the control station (A) was located outside the cave on an old railway bridge 8 m above sea level. The light spectrum was determined using a portable spectrophotometer and the spectroscopy software Spectrasuite (Ocean Optics

Inc., Orlando, FL, USA). Light intensity was measured with a direct quantum sensor using LI-250A light meter equipped with the LI-200SA Pyranometer Sensor (LI-COR Bioscience, Lincoln, NE, USA), as described in Abu-Ghosh et al. (2015). During the measurements, we placed the two sensors parallel to the sea on a permanently raised platform directed 270° to the north-west, following the natural direction of the main grottos along the Rosh HaNiqla fault plane. Light

measurements were taken in 2-h intervals from 10:00 hours to closing hours at 16:00 hours in the winter and 18:00 hours in the summer. The light spectrum was divided into the ultraviolet (UV; UV-B: 280–315 nm; UV-A: 315–400 nm) and infrared (IR: 780–1200 nm) wavebands within the PAR, as well as violet (400–440 nm), blue (440–490 nm), green (490–570 nm), yellow and orange (570–620 nm), and red (620–780 nm). To obtain the inside/outside of grotto ratios, the waveband intensities at each station, season, and hour were divided by the waveband sum at each station, season, and time. This graph enabled us to determine the proportion of each waveband from the full light spectrum. We then divided the proportion of each waveband inside the grottos by the waveband proportion outside the grottos to detect the changes in waveband ratios when light entered the cave. Temperature and relative humidity were measured 7 times a year using a data logger (MultiLog DB-526; South Africa) during daylight hours (from 8:00 hours to 19:00 hours).

In-situ growth monitoring

To study algal growth in the cave in relation to different seasons, we applied a method developed by Murphy et al. (2005) for coastal EPC monitoring via the reflectance of chlorophyll *a*. Accordingly, we measured the chlorophyll *a* concentration in the field for 6 months across the study period using a portable spectrophotometer (Cary-300 UV–Visible Spectrophotometer; Agilent Technologies, Santa Clara, CA, USA) at sites of red and green EPC biofilms. Chlorophyll *a* concentration was estimated by dividing the reflectance intensity at 670 nm/750 nm wavelengths, whereas the background light was calibrated at 750 nm.

Taxonomy

The morphological features of EPCs were defined for taxonomic identification. Red and green rock samples from EPC biofilms were collected from inside the cave using a hammer, chisel, and scalpel. The samples were then transported to the laboratory in an icebox where, upon arrival, they were either immediately analyzed or refrigerated at 4 °C in the dark. For light microscopy examination, we gently scraped the samples off the stone, placed them on glass slides, and observed them under a fluorescence microscope (Axio Imager Z1; Carl Zeiss Microscopy GmbH, Jena, Germany) and the Nikon ECLIPSE E200 microscope (Nikon Instruments Inc., Melville, NY, USA) at magnifications ranging from 100× to 2000×. The microscopes were managed using AxioVision software (Carl Zeiss Microscopy GmbH) and equipped with two cameras, an Olympus E-450 camera (Olympus Corp., Tokyo, Japan) and an OMAX A35100U camera (OMAX, China). Microscopic examinations were carried out at Bar-Ilan University and Haifa University. For

the scanning electron microscopy (SEM) analysis, rock samples were first placed in a special sample holder, covered in Carnovsky fixative solution, and left at room temperature for 5–8 h in the dark. The samples were then washed 3 times with phosphate-buffered saline (PBS), followed by emersion in a graded series of increasing ethanol concentrations and a final wash with Freon. The next day, samples were coated with gold and observed using a high-resolution electron microscope equipped with a newly developed electron column with a monochromator (Magellan 400L SEM-FEG [field emission gun] (FEI Co., Hillsboro, OR, USA), at Bar-Ilan University. To determine the taxonomic position of organisms, we used ecomorphological and polyphasic approaches, i.e., genotypic, chemotaxonomic, and phenotypic methods (Prakash et al. 2007; Schildkraut et al. 1961).

The algal community was also investigated using an Olympus BX50 light microscope equipped with a Nomarsky system (Olympus Corp.) and photographed using a Leica MC170 HD digital camera (Leica Microsystems, Wetzlar, Germany). Marine red algal species were examined for the appearance and division pattern of tetrasporangia, conceptacle size and shape, presence of thalli, and cell dimensions. Marine specimens with shallow conceptacles (up to 80 µm deep) and tetrasporangia with non-parallel divisions were designated as *H. rubra* based on morphological features (Abbott and Hollenberg 1976; Irvine and Chamberlain 1994), with a comparison of molecular properties (Sherwood and Sheath 1999, 2000). Cyanobacterial species on the cave walls were identified after cultivation on BG-11 media for 2 weeks (Guiry and Guiry 2022); other organisms, such as mosses, were identified in cultures.

DNA extraction, amplification, and sequencing from red-colored samples

Genomic DNA was extracted from the red-colored specimens collected at station A using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. DNA was eluted into 50 µl of nuclease-free water and stored at –20 °C. For amplification of the gene encoding the large subunit of ribulose-bisphosphate carboxylase (*rbcL*), which is a chloroplast marker, we designed the forward primer Hil-1 (*rbcL* 5'-AGATATGCGTATACCAGTAGCTTAT-3') and reverse primer Hil-1 (*rbcL* 5'-CACGATTTGCAGTTGCTCCA-3'). DNA concentration was measured using a NanoDrop UV–Vis Spectrophotometer (Zhengzhou Labao Instrument Equipment Co., Ltd., China). The optical density of the solution was measured at a wavelength characteristic of DNA (260 nm), in micrograms per milliliter (µg/ml). The ratio of absorption at wavelengths of 260 and 280 nm (A₂₆₀/A₂₈₀) was used to assess the purity of the preparation. Pure DNA has an A₂₆₀/A₂₈₀ ratio of about 1.8, and a sample of RNA without impurities has an A₂₆₀/A₂₈₀ of about 2. Thus, the criterion for selecting the

samples analyzed in the present study was the threshold of $A_{260/280} = 1.8$. PCR amplification was performed in a 50- μ l reaction volume containing 2 $\times$ PCRBIO Taq Mix Red (PCR Biosystems Ltd, London, UK), in a 96-well Veriti TM Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA), resulting in an approximately 849-bp PCR product. The PCR cycling conditions were 35 cycles of 95 °C for 120 s, 58 °C for 15 s, 72 °C for 30 s, with an extension at 72 °C for 420 s and a hold at 4 °C. PCR products were purified using Wizard I SV Gel and PCR Clean-up System (Promega Corp., Madison, WI, USA), and were sequenced in both directions at HyLabs (Hy Laboratories Ltd, Rehovot, Israel) with the same primers as used for PCR. All PCR products were analyzed by electrophoresis in 1% agarose gel.

Phylogenetic analysis of red alga

Phylogenetic analysis was based on the chloroplast marker *rbcL* gene (849 bp) sequences. Approximately 100 other sequences were chosen from GenBank for the phylogenetic analyses. Sequences were picked based on: (1) similarity to our sequences of *Hildenbrandia* as determined by BLAST searches; and (2) sufficient base length of the sequence. The *rbcL* gene sequences were aligned manually on Microsoft Word (Microsoft Corp., Redmond, WA, USA).

Hildenbrandia distribution and proliferation in Rosh HaNiqra cave

A rock surface in the red zone of station B (Balcony of Colors) dominated by the epilithic *Hildenbrandia* rhodophyte was chosen to study the seasonal effects of distance from the water line on the areal cover of that alga. For this purpose, photographs were taken once a month for 1 year beginning in May 2022. At that location, three sampling points were selected at different distances from the water: 50, 100, and 200 cm. The photographs were analyzed using ImageJ software to calculate the percentage area of the algal coverage of the surface (Xiong et al. 2019).

Statistical analysis

Student's t-test of the light spectrum in the grotto revealed the light composition in the Rosh HaNiqra grotto wall communities. A correlation analysis was carried out using the JASP statistical program (Love et al. 2019).

Results

Natural light regime inside the cave

Light measurements revealed that the light intensity inside the Rosh HaNiqra midlittoral cave ranged from 0.5 to 2.5% of the total outside light intensity (Fig. 2). The percentage of internal light recorded depended on the amount of direct light entering the cave, which varied according to the season and the time of day. The results showed that relatively more direct light entered the cave when the sun was setting (Fig. 2), documented mostly in the summer as a slow rise in intensity until two jumps in light intensity at 16:00 hours and 18:00 hours (Fig. 3). In the winter, light intensities were relatively constant, with an increase in the afternoon and a decrease towards sunset (Fig. 3). The results from the control station outside the cave showed that PAR comprised a steady 80% of the whole light spectrum (280–1200 nm) in all seasons and at all hours, with a constantly high green waveband, an increase in the red waveband, and a decrease in the blue waveband at sunset (Fig. 3). These findings represent the normal distribution of sunlight and agree with those reported in other studies on the light spectra of midlittoral caves (Wakiyama and Ohba 1997; Mayer et al. 2016). However, changes in PAR (range 400–700 nm) inside the cave were far more significant ($p < 0.001$) than those reported in other studies, accounting for 55–80% of the whole light spectrum (280–1200 nm) in the summer compared to 60–65% in the winter. Within the PAR range, there was also an increase in the red waveband towards sunset inside the cave, similar to the red enrichment observed in the control station (Fig. 3).

Taxonomy of red algae

From 2019 to 2022, we surveyed the Mediterranean coast in Israel, ranging from Ashdod in the south to Rosh HaNiqra in the north, for *Hildenbrandia* by measuring the chlorophyll *a* concentration of red and green rock samples from EPC biofilms. We found this alga only in Rosh HaNiqra and the Yam Rosh HaNiqra area. *Hildenbrandia* species were defined based on morpho-ecological features and DNA barcoding of the *rbcL* gene sequence. Morphological characteristics used to define the red-colored periphytonic crusts as *H. rubra* were tetrasporangiate type of division, thallus thickness, conceptacle size and shape, presence of thalli, and cell dimensions. Marine specimens exhibiting shallow conceptacles (up to 80 μ m deep) and tetrasporangia with non-parallel divisions are indicative of *H. rubra* (Sherwood and Sheath 1999, 2000).

Morpho-ecological and molecular analysis defined *H. rubra* from the epilithic community of the Rosh HaNiqra

Fig. 2 Average light intensity and spectral light distribution outside the cave **a** compared to inside the cave **(b)**. The gray shaded area is the sum of all the wavebands. Colors of the bars represent the light distribution of the different wavebands. *IR* Infrared, *UV* ultraviolet

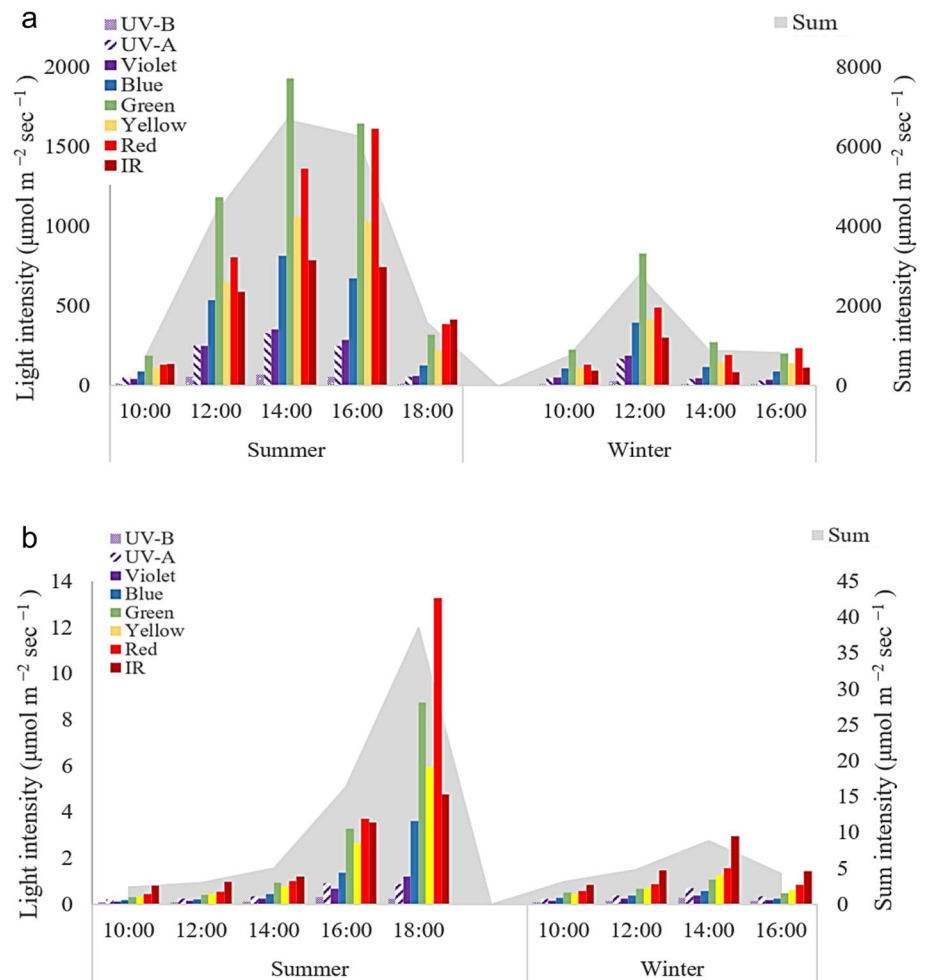
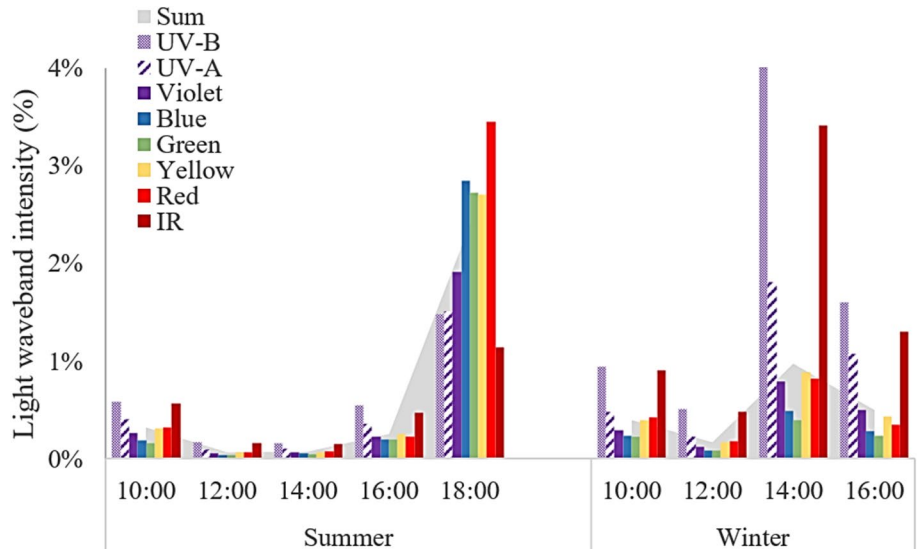


Fig. 3 Average ratio of wavebands inside the cave divided by the wavebands outside. The gray shaded area is the sum of all the wavebands. Colors of the bars represent the light distribution of the different wavebands. *IR* Infrared, *UV* ultraviolet



grotto that was found in station A, along with cyanobacteria and moss protonema (Fig. 4). Species that occupied the wet wall and stones (Fig. 4a, b) have seasonal preferences

regarding the development of reproductive structures. We found the most critical morphological characteristic for *H. rubra* definition to be the irregular cell division of

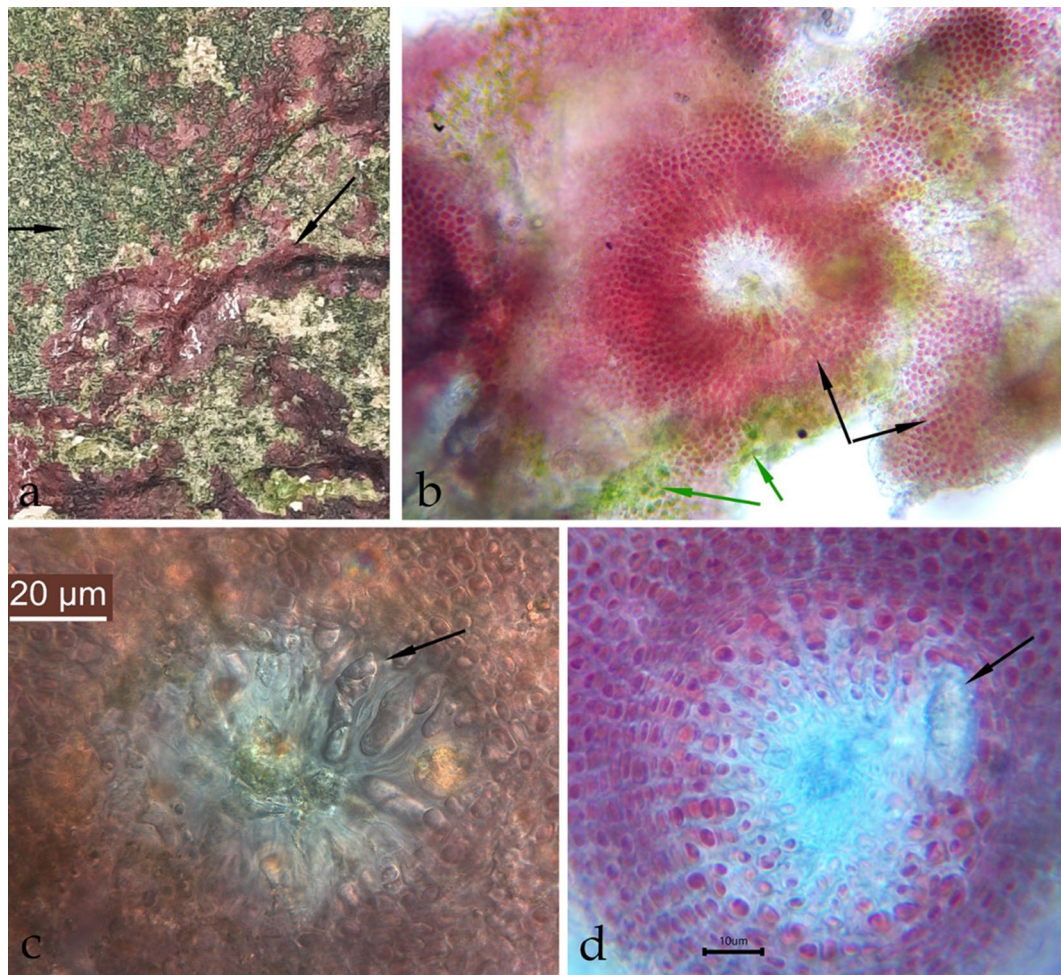


Fig. 4 *Hildenbrandia rubra* found in the cave of Rosh HaNiqla. Epilithic community at the site. **a** *Hildenbrandia rubra* (right arrow) and protonema of mosses (left arrow). **b** *Hildenbrandia rubra* sporangial conceptacle producing tetraspores (black arrows) and protonema of

mosses (green arrows). **c** *Hildenbrandia rubra* conceptacle with tetrasporangia (arrow). **d** *Hildenbrandia rubra* parenchyma near conceptacle with tetrasporangia (arrow)

tetrasporangia (Fig. 4c). Other characteristics include species characteristics, such as parenchyma, diameter of the conceptacles, and pigment content (Fig. 4b–d).

The data obtained from the *rbcL* sequencing of the red-color samples collected from station A in the Rosh HaNiqla grotto was inserted into the National Center for Biotechnology Information (NCBI) database for comparison with known sequences of species. Comparison of the chloroplast gene sequencing product of the sample with 16 other populations showed a similarity of > 83.69%. Sequences of the Rosh HaNiqla sample (outlined in yellow in Fig. 5) were most similar to those of *H. rubra* populations from the USA and Mexico with a similarity of > 95.09% (the last branch of the tree with population numbers 1, 2, 3, and 4) (Fig. 5). Altogether, based on the similarity of the morpho-ecological characteristics, we concluded that the red-colored samples collected from

station A on the Rosh HaNiqla grotto are representative of the marine red algae *H. rubra*.

Red algae ecology

Our survey determined that the best site for a red algae-dominated community was the northern-most site, namely, Rosh HaNiqla. The optimal growth conditions for these rare red algae are a shaded and clear water environment. These conditions are not found along the entire coast studied because humans have greatly modified the coast and because of an oil accident in May 2022, which affected all of coastal habitats of Israel up to the northern border; these two factors explain the absence of *H. rubra*. The Rosh HaNiqla caves protected the water from the effects of oil pollution and also provided a shaded environment, thereby providing an optimal environment for this red alga. It is notable that



Fig. 5 Maximum likelihood tree of the *rbcL* gene of *Hildenbrandia rubra*. Bootstrap values > 83.69% similarity are included. The scale bar indicates 14% sequence divergence. Sample 3245_Hildenbran-

dia_rubra_country_Israel_Rosh_HaNiqlra_rbcL_gene is highlighted in yellow. Sequences were obtained from living material. The taxon names and NCBI GenBank numbers are provided

seasonality is important for the growth of *H. rubra*. Based on our results, the best season for the formation of numerous conceptacles is from the beginning of autumn up to the beginning of winter (or from the end of September up to the end of December), depending also on the ambient air temperature at the time of the year. After 48 h, *H. rubra* showed maximum efficiency under low light intensities, with a significant acclimation to low light and maximum photosynthetic potential, changing from 50 to 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Finally, *H. rubra* fully recovered, exhibiting high photosynthetic potential without long-term effects. These tests show that *H. rubra* is extremely suitable for the abiotic conditions of the midlittoral cave.

***Hildenbrandia* distribution and proliferation in Rosh HaNiqlra cave**

Comparison of all sample images revealed that the largest surface area covered by *Hildenbrandia* occurred in the site farthest from the water (Fig. 6), except for during the winter, probably because more intense cold-water waves wash the rock surface near the water in the winter, affecting the

ability of the algae to proliferate. In contrast, there was no significant difference in algae coverage in the two other sites across the year. The distribution of algae was the lowest in the winter in all three sites, gradually increasing in the spring and summer (Fig. 6), suggesting that the higher temperature in this season (Fig. 6) and avoidance of the waves positively impacted the algae distribution in the cave.

Biodiversity of algae samples

To identify the red algal species present in the cave, algal samples were studied under the microscope and DNA was extracted from the biosamples and subjected to *rbcL* gene library preparation. The findings revealed distinct dominant algal species across the sampled locations. Specifically, the red algal species *H. rubra* was identified as the predominant alga in the “Balcony of Colors” at station B within the cave, while the green algae *Blidingia dawsonii* and *Chlorella* spp. were more prevalent outside the cave at station A (Fig. 7).

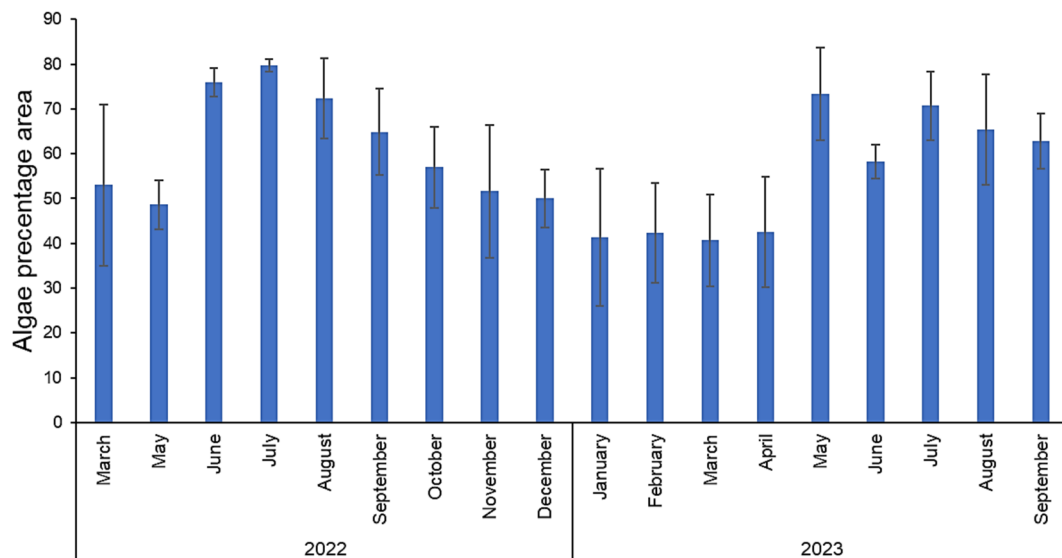


Fig. 6 *Hildenbrandia* distribution and proliferation in the Rosh HaNiqlra cave across the study period

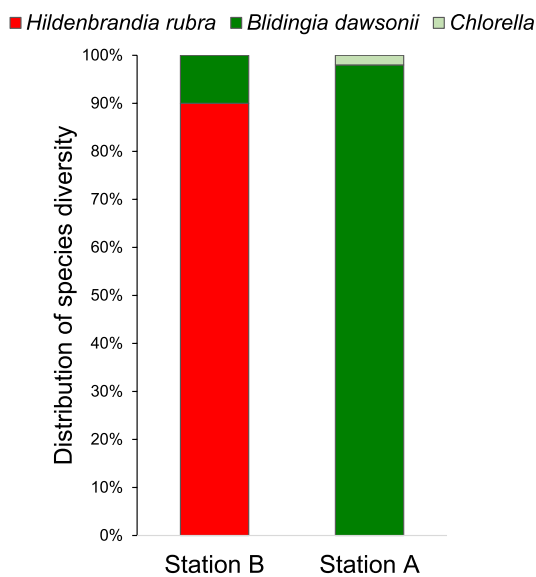


Fig. 7 Biodiversity of algae samples from the Rosh HaNiqlra cave

Discussion

In this study, we investigated the ecological relationship between the red and green epilithic algal communities and the Rosh HaNiqlra cave's surrounding environment by taxonomically identifying the upper midlittoral epilithic algal community and quantifying and qualifying the natural light in Rosh HaNiqlra cave, Israel.

Light intensity and spectrum in the Rosh HaNiqlra cave

Our findings show that the light intensity within the Rosh HaNiqlra midlittoral cave ranges from 0.5% to 2.5% of that of the outside light, with variations due to seasonal and daily changes, with the light intensity particularly higher during summer at sunset. PAR was found to account for 80% of the total light spectrum regardless of season or time of day outside the cave. However, PAR fluctuates inside the cave, comprising 55–80% of the total light spectrum in summer and 60–65% in winter. Our observation that light in the cave at sunset was predominantly in the red wavebands suggests an increased reflection of longer wavelengths, which is consistent with the findings of previous studies reporting that 5–10% of the light that reaches the water's surface at noon is reflected, with longer wavelengths being relatively more reflected (Abu-Ghosh et al. 2016; Zhou et al. 2017). In summer, direct light enters the cave due to the location of the sunset directly in front of the cave opening, thereby increasing surface reflection. This can account for the high amount of PAR in the cave at sunset, as a smaller angle ($< 90^\circ$) of sunlight incidence before and after midday causes more reflection (Wakiyama and Ohba 1997). Also, light distribution is far more enriched in longer wavelengths, creating higher proportions of UV, red, and IR dominance inside the cave, as opposed to the regular distribution of light wavebands, which is highest in green (green > red > blue) wavebands outside the cave. The high amount of PAR in the cave during sunset is attributed to direct sunlight and increased surface reflection, as PAR in shaded areas is a function of

sea surface light reflection and decreases with distance from the sea (Wakiyama and Ohba 1997).

Similarities have been shown between light patterns in the shade in tree canopies and those in midlittoral cave habitats (Norton 1971; Wakiyama and Ohba 1997): illumination through diffused/indirect light rapidly decreases PAR, allowing the dominance of infrared and ultraviolet. Moreover, shorter wavebands are more intense when direct light enters a cave (Norton 1971). In contrast, our findings show that red wavebands are most intense inside the Rosh HaNiqra cave at all hours and seasons, with higher proportions of UV, red, and infrared (1.6- to fourfold) light compared to light outside the cave, which decreased only during the summer at sunset. This suggests a unique light distribution inside the cave that is influenced by specific reflection and refraction conditions. The red waveband's dominance could be associated with the increased reflection of longer wavebands of the sea surface once the diffused sunlight hits the water surface. The difference between reflection into the cave or refraction to the water column is generally dependent—but not limited to—the frequency of the light wave and the sun's altitude. Hence, the decrease in IR during the summer at sunset could be due to the exceptionally low angle of the sun, the cave's narrow opening, which allows less light to enter the cave directly, and higher amounts of light reflection and refraction. At sunset, red and IR wavebands enter the water; however, 90% of infrared is absorbed in the first 1–2 m of the water column (Iluz 1998; Dishon et al. 2012). This phenomenon causes increased absorbance of the IR waveband in the water column, leaving the red light more dominant at sunset. The unique light environment of the Rosh HaNiqra cave, particularly the enrichment in longer wavelengths, may create niche opportunities for specialized algal species. This phenomenon has been observed in other low-light marine environments, such as the deep chlorophyll maximum studied by Falkowski and Laroche (1991).

In addition, the high humidity in the summer at sunset and during the afternoon, which creates conditions in which the IR waveband is efficiently absorbed and transformed into heat, may also explain the decrease in ambient IR in the summer at sunset. High red and IR wavebands inside the cave, in contrast to the light spectrum outside, result in the midlittoral cave acting as a natural long-pass filter. Long-pass filters attenuate shorter wavebands and transmit longer wavebands over the active spectrum. Such filtration could result from atmospheric scattering, represented by Rayleigh scattering. Under these conditions, scattering occurs primarily through sunlight's interaction with randomly located air molecules, where shorter (blue) wavelengths are scattered more strongly than longer (red) wavelengths (Kirk 1994). This can explain why more IR and red wavebands manage to enter the cave, rendering them the dominant wavebands most of the time inside Rosh HaNiqra's cave. Interestingly, the

relative amount of UV radiation relative to the total amount of radiation inside the cave and the relative amount of UV radiation outside the cave is also considerably higher inside than outside. This trend can be found in the summer at sunset and at the highest light intensity in the winter when the sun does not set in front of the cave but south of the entrance. Therefore, at the peak of winter illumination, the light may enter the cave from the water column or the indirect light. However, in the summer at sunset, as more light enters the cave through the water column, direct light enters the cave. Our results depict a unique phenomenon occurring during the summer at sunset, where the sea surface acts like a mirror, reflecting the sunlight into the cave from the water surface through the air. Moreover, the plain walls of the cave allow the light to reflect from their surface, increasing the light intensity inside the cave but not outside.

Algal zonation

In this study, we identified the red alga *H. rubra* in the Eastern Mediterranean for the first time. This common epilithic alga has been reported in Turkey, Lebanon, Egypt, and South Arabia (Lakkis and Novel-Lakkis 2000; Kanaan et al. 2014; Ansari 2016). However, it has only been documented once in Israel, 40 years ago in a submerged reef south of Mikhmoret (Lundberg 1996). We report here the presence of *H. rubra* at a location above sea level in Israel for the first time. The growing shortage of algal taxonomists, particularly those on coastal EPC, likely contributes to the sparse records of *H. rubra* in Israel and the limited algal data in the eastern Mediterranean, and may also explain the absence of recent reports of *H. rubra*, even after the 2018 marine biological survey (BioBlitz). However, during our revisit to the south reef in Mikhmoret in August 2016, we identified *Hildenbrandia* spp. at the documented location (data not published).

In the present study, red algae were identified using both morphological features and DNA sequencing methods. Our findings indicate that *Hildenbrandia* spp. isolated from Rosh HaNiqra cave is very sensitive to water pollution, requiring a clear, shaded marine water environment to thrive. We observed higher reproduction patterns in the autumn and winter seasons. Future studies should include an initial taxonomic survey of the coastal EPCs from the Israeli shoreline to map key EPC habitats and uncover unlisted algal communities. Such studies are crucial in the context of global and local climate changes, as well as infrastructures and marine reserve plants on the Israeli coast. Our analysis revealed that the red samples primarily consisted of *H. rubra* and moss protonema. We identified chlorophytes belonging to the *Ulvophyceae* class but were unable to identify the samples to the species level. In two samples, we identified the calcifying rhodophyte *Phymatolithon lenormandii* at the cave entrance and, on two occasions, in association with *H. rubra*.

The upper midlittoral cave is mainly covered with mixed EPCs, predominantly *H. rubra* and mosses, interspersed with cyanobacteria.

A review of over 50 surveys involving *H. rubra* revealed that the alga typically inhabits water at depths ranging from +5 to −20 cm (Relini and Giaccone 2009), where red wavelengths of light are more abundant. This spectral enrichment might explain the link between *Hildenbrandia* and midlittoral caves. The high enrichment of blue and green wavebands enables algae to optimize light use through pigments like chlorophylls *a* and *b*, which may explain the success of red macroalgae under the low-light conditions of midlittoral caves. Studies have shown that midlittoral caves are common habitats for *H. rubra* (Dellow and Cassie 1955; Connor et al. 2003a, b; Guiry and Guiry 2016; Tyler-Walters 2016), although the optimal habitat of this algal species has not yet been pinpointed (Kaehler and Williams 1996). Our results indicate that Rhodophyta possess photosynthetic pigments such as phycoerythrin and chlorophyll *a*. Phycoerythrin allows algae to photosynthesize using green and blue wavebands, enabling *H. rubra* to thrive under the conditions of low-light intensities found in midlittoral caves. We conclude that *H. rubra* benefits from the optical properties of phycoerythrin and chlorophyll *a*, as these pigments make red and blue wavelengths more available in the cave environment. This adaptation allows *H. rubra* to grow outside its usual habitat in the littoral zone, thriving in midlittoral caves with similar light conditions (shade) but less competition, namely, the midlittoral cave. *Hildenbrandia rubra*'s high tolerance for the abiotic fluctuations in temperature, pH, dryness, and low light enables its survival above sea level in midlittoral caves worldwide, extending its habitat beyond the more comfortable infralittoral zone.

The distribution and proliferation of *Hildenbrandia* within the Rosh HaNiqra cave exhibited notable patterns. For most of the year, with the exception of winter, the largest algal surface coverage was found at the site closest to the water's edge. This seasonal variation can be attributed to the intense cold-water waves washing over the rock surface near the water during winter, potentially hindering algal growth and proliferation. Interestingly, the two other sampling sites within the cave did not show significant differences in algal coverage throughout the year. However, a consistent trend was observed across all three sites, with the lowest algal distribution during the winter months and a gradual increase in coverage during the spring and summer seasons. This pattern suggests that the higher temperatures and reduced wave exposure during the warmer months create favorable conditions for algal growth and distribution within the cave environment.

The percentage of algal coverage on the surface indicates that communities in the Rosh HaNiqra Cave are closely linked to the environmental conditions of the sampled point.

Specifically, EPCs changed their color on the stones' surface from red, dominated by *H. rubra*, to green, where the EPC was enriched by moss protonema. The cyanobacteria-dominated EPC, containing a mixture of potentially new-to-science species belonging to the genera *Chroococcidiopsis* (family Chroococcidiopsidaceae) and *Pleurocapsa* or *Myxosarcina* (family Pleurocapsaceae) along with moss protonema (kingdom Plantae, phylum Bryophyta) was responsible for the black and dark blue-green colors observed on the walls of the cave at station A.

The diversity and richness of the algal species decreases from the entrance to the far end of the cave, a trend that was found to be primarily influenced by light intensity, with a positive correlation with increasing darkness. The availability of water had a lesser effect due to the consistently high humidity in the air. The decrease in temperature also plays a role in the poor taxonomic algal variety in the cave. Kim and Garbary (2006) reported that *H. rubra* is one of the most physiologically tolerant species of the intertidal crust algae. In their laboratory experiments, *H. rubra* thalli were exposed to temperatures between −17 °C and 27 °C, from fully hydrated to extremely desiccated, from full seawater to 4 psu and back again over a period of 13 days, without any apparent long-term effects to the photosynthetic apparatus, and no significant difference in photosynthesis. Overall, a reduction from 'full' to 'reduced' salinity may result in the loss of a proportion of the EPC in the upper intertidal zone, while *H. rubra* is probably resistant to this pressure.

The findings of this study enhance our understanding of the environmental factors influencing algal biodiversity and distribution in coastal cave ecosystems. The presence of distinct dominant algal species in different cave regions suggests niche partitioning and adaptation to specific microhabitats within these unique environments. Additionally, the observed seasonal variations in algal coverage highlight the importance of considering temporal dynamics and environmental fluctuations when studying cave-dwelling algal communities. It is important to note that this study focused on a specific cave system, and algal diversity and distribution patterns may differ in other coastal cave environments due to variations in environmental conditions, geological features, and geographic locations. Future research could explore the algal communities in additional cave systems, incorporating more comprehensive sampling strategies and environmental data to further elucidate the drivers of algal biodiversity and distribution. Also, investigating potential interactions between the dominant algal species and other organisms within the cave ecosystem, such as invertebrates or microbial communities, could provide insights into the ecological roles and relationships within these unique habitats.

In conclusion, this study sheds light on the algal biodiversity and distribution patterns within the Rosh HaNiqra cave, highlighting the influence of environmental factors and

seasonal variations on these communities. The findings contribute to our understanding of coastal cave ecosystems and provide a foundation for further exploration of the intricate relationships between algae and their cave environments.

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Author contributions All the authors have contributed to the manuscript in various ways, such as problem identification, sampling and analysis, interpretation, writing, editing, and proofreading. DI, SB, ZD: conceptualization, methodology and coordinated sampling. SA, DM, SB, EG, EC, and AA: fieldwork and sample analysis. SB, EC, and EG: DNA studies. SA and DI interpreted the results and wrote and edited the manuscript.

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Declarations

Conflict of interest The authors have no conflicts of interest to declare.

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Consent to participate Not applicable.

Consent for publication All the authors have given their consent for publication and no data of any person included in the manuscript.

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