Environmental drivers of phototrophic biofilms in an Alpine show cave (SW-Italian Alps)

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Abstract

The proliferation of *lampenflora* is a major threat for the conservation of show caves, since phototrophic organisms are responsible of physical, chemical and aesthetic damage. In this paper we examined the environmental factors influencing the presence and the growth of the three main photosynthetic groups composing autotrophic biofilms (cyanobacteria, diatoms and green algae) in the Bossea show cave (SW-Italian Alps). Presence and primary production of the three considered photosynthetic groups were detected with the BenthoTorch®, an innovative instrument for *in situ* measurement of chlorophyll *a* concentration. By means of different techniques of regression analysis, we highlighted the response of the three photosynthetic groups to different environmental factors. Illuminance proved to be the main factor driving both the probability of presence and the growth of the three groups inside the cave. The presence of seeping water on the substrate and the distance from the cave entrance proved to play an important role in determining patterns of colonization. By means of GIS techniques, we provide several thematic maps, highlighting the growth pattern of the three examined photosynthetic groups within the Bossea show cave. The same approach may apply to other show caves, aiming at providing suggestions for the cave

management (i.e. cleaning of the cave walls and positioning of artificial lights) and reduce impact caused by tourism.

Keywords

Lampenflora, show cave, primary production, BentoTorch®, GLMM, ZIG

Highlights

- We used a PAM fluorimeter to measure phototrophic biofilms in a show cave
- We modelled the environmental factors influencing the growth of autotrophic biofilms
- Illuminance, moisture and distance from the entrance proved to be the main factors
- We produced thematic maps illustrating our results
- We provide suggestions for cave management

1. Introduction

In natural conditions, cave ecosystems are extremely oligotrophic habitats characterized by the absence of light and low floristic and faunistic diversity (Holsinger 1998). Primary production in caves is generally limited to the cave entrance, where phototrophic communities are able to exploit natural light. When caves are illuminated for touristic purposes (the so called “show caves”), primary production dramatically increases as a consequence of the installation of artificial lighting systems (Albertano 2012). Producers in show caves are mostly epilithic prokaryotic and eukaryotic microorganisms of various taxonomic groups, including cyanobacteria (Cyanophyta), diatoms (Bacillariophyta) and green algae (Chlorophyta) developing on the surface of speleothems (Aley 2004; Roldán & Hernández-Mariné 2009). Indeed, environmental impacts in show caves generally relate to the opening of the cave to public and to the presence of artificial lights. Moreover, tourists in caves alter the natural microclimatic conditions in terms of relative humidity, air temperature, CO_2 concentration (Cigna 1993; Albertano 2012) and possibly bring external organisms inside the cave, having potential repercussions on the entire subterranean ecosystem (Saiz-Jimenez 2010).

According to several authors (Aley 2004; Mulec & Kosi 2009; Cigna 2011) autotrophic microorganisms also survive in dark areas, but, indeed, the presence of artificial lights, especially in the dark zone of the caves, determines very favourable conditions for their growth. The absence of a photoperiod combined with an almost constant temperature and a high relative humidity allows autotrophic organisms to grow better in the deepest zones than at cave entrances (Mulec et al. 2008). Moreover, the limited air circulation in caves favours an increase of particle concentration and the chances of settlement on surfaces (Albertano 2012). Photosynthetic unicellular microorganisms grow as components of biofilms (Hoffmann 2002), forming thick green, brown or greyish patinas on cave walls and other speleothems (Hoffmann & Darienko 2005; Bruno et al. 2006, 2008; Lamprinou et al. 2012). Secondarily, mosses or ferns may also colonize the same substrates. The community of autotrophic organisms developing in caves next to artificial lights constitute the so-called *lampenflora* (Dobat 1963).

Several authors reported about the impact of *lampenflora* in show caves (Northup & Lavoie 2001; Hoffmann 2002; Albertano et al. 2003). In particular, the development of photosynthetic biofilms cause aesthetic, physical and chemical damage. For instance, while the exopolymeric substances (EPSs) produced by cyanobacteria and algae ensure the protection of microorganisms from desiccation, at the same time they induce the adsorption of cations and dissolved organic molecules from the mineral surface causing the deterioration of the substrate (Albertano et al. 2003). When *lampenflora* biomass becomes available to cave-dwelling organisms, it may cause alterations of food chain of the subterranean ecosystem. Such changes may affect dramatically subterranean specialized species (Mulec 2012).

For these reasons, specific knowledge of the ecology of *lampenflora* is needed. Recently Albertano (2012) and Falasco et al. (2014) reviewed the main environmental factors influencing the colonization, the growth and the diversity of cyanobacteria and diatoms in caves respectively, pointing out the major role played by light and humidity. Moreover, other factors such as water and air circulation, the distance from the cave entrance, mineral and nutrient availability, substrate roughness and pH may play an important role in photosynthetic biofilms growth.

In our paper, we examine the influence of environmental factors in determining the colonization and the primary production of the three main components of photosynthetic biofilms, namely cyanobacteria, diatoms and green algae (Dayner & Johansen 1991; Hoffmann 2002; Smith & Olson 2007; Mulec 2012), in the Bossea show cave (SW-Italian Alps). In particular, we applied regression models to investigate the environmental parameters affecting both the probability of presence of the three groups and their concentration of chlorophyll *a* taken as proxy for biofilms primary production.

Several studies have examined the relationship between the diversity of the photosynthetic community and the cave environment (Vinogradova et al. 1998; Roldán et al. 2004; Lauriol et al. 2006; Roldán & Hernández-Mariné 2009; Martínez & Asencio 2010), but only few focused specifically on primary production and photobiota density (Mulec et al. 2008; Roldán & Hernández-Mariné 2009; Borderie et al. 2014).

Specifically, we hypothesize that: i) illuminance is the main environmental factors influencing both presence and primary production of cyanobacteria, diatoms and green algae; ii) cyanobacteria, diatoms and green algae show different responses to the considered environmental parameters.

2. Materials and Methods

Study area

The Bossea show cave is part of a karst system developed in a sub-basin of the Corsaglia watershed, located in the SW-Italian Alps (municipality of Frabosa Soprana, Province of Cuneo, NW-Italy).

The Bossea cave is particularly rich in water since it is crossed by a subterranean river flowing inside the cave with a flow rate ranging from 50 (winter) to 1200 l/sec (spring). The subterranean stream develops as part of a stream basin of approximately 6 km^2, over an altitudinal range of nearly 700 m and a maximum length of 5 km (calculated from the point where the subterranean stream flows out of the cave to the highest peak of the sub-basin).

The cave is set in a narrow belt of limestone and dolomitic limestone imposed upon permo-carboniferous rocks (quarzites and porfiroids). The most common speleothems in the Bossea cave are flowstones, composed by microcrystalline secondary calcite. Other kind of substrates (micritic calcite and porfiroids) are rare in the cave and limited to rock-slides and emerging bedrock.

The cave has a total planimetric development of 2,800 m and an ascending structure. It opens at 836 m asl and reaches 1,040 m at its highest point.

From a meteorological point of view, the Bossea cave can be considered a “hot-air” trap. Because of its ascending structure, it is affected by air convective movements strictly related to the different densities of internal and external atmospheres. During summer the air inside the cave is colder and denser than outside, thus flowing on the floor along the conduits, exiting from the lower part of the cave entrance. This air flow creates a depression into the upper part of the cave, and then at the

same time an identical amount of warmer external air enters from the upper part of entrance. On the other hand, during winter the internal air is warmer and less dense. The air flow stops and the cave becomes a “hot-air” trap (Badino 2004, 2010).

The present entrance of the cave corresponds to an old spring of the subterranean river that was active when the level of the subterranean stream was higher. Erosive processes (both inside and outside the cave) modified the flow of the water and lowered the level of the stream in the course of time of nearly 30 m. Nowadays the subterranean stream flows outside the cave directly in the Corsaglia river through a series of submerged syphons. The fossile riverbed and its opening represent nowadays the only direct connection of the cave with the external.

The cave was firstly explored in 1850 and opened to public in 1874. With 150 years of tourist frequentation, the Bossea show cave is the oldest Italian cave open to public. The current touristic flow is estimated in 12,000 visitors per year (Dal Pozzolo et al. 2013). Tourists have access to the cave via the narrow corridor (the above-cited fossile riverbed) of nearly 100 m length, which is often used for contemporary art expositions. After the corridor and along the touristic pathway, visitors have access to a consecutive series of vast rooms such as the Sala Garelli of 100 m long, 60 m wide and 40 m high. The touristic pathway ends at the Ernestina Lake at 949 m asl, nearly 1 km away and 113 m higher than the entrance. After the Ernestina Lake the cave is closed to public. A long active canyon of 3-6 m wide and up to 40 m high follows, ending in a system of submerged syphons, which were partially explored by speleo-divers (Gregoretti 1991).

Artificial light systems were first installed in 1948 all along the touristic pathway. At the present days, lights consist of halogen lamps placed in the nearby of speleothems of particular interest. Moreover, a number of neon lamps is placed along the touristic pathway (20-40 cm from the ground).

Lamps are turned on during visits. Several illuminated surfaces (especially stone waterfall formations, stalactites, stalagmites and columns) are covered by green-brown or greyish patinas, especially those directly illuminated by the lamps. In order to remove patinas, cleaning operations

are carried out irregularly once a year, especially on walls covered by flowstone next to the lights, where patinas are consistent and visible. Sodium hypochlorite 5% is used for this purpose.

Sampling design

We selected 42 illuminated walls covered by flowstone (plots), distributed at a progressive distance from the cave entrance and at different distances from the touristic pathway. No plots were placed inside the touristic pathway (minimum distance 1 m). At each plot we selected 3 sampling points: close (< 50 cm), at intermediate distance (50 cm - 2 m) and far from the light source (2-5 m), for a total of 123 sampling points (41 plots x 3 points for each plot). Each sampling point consisted in a circle of 10 cm diameter. All sampling plots were illuminated with white halogen lamps Philips 200W and 300W, with a color temperature of 3000 K and 2900 K respectively. These bulbs are essentially black-body radiators, with maximum emission in the IR range, at wavelength of 1000 nm. The spectrum in the visible range is shown in Fig. 1a, superimposed on the typical absorption peaks of chlorophyll-*a*. Fig. 1b shows the chlorophyll-*a* energy absorption efficiency within this light spectrum, with an absorption peak at 660 nm. Neon lamps along the touristic pathway were considered uninfluential since no sampling plot was placed inside or in close proximity of the touristic pathway.

All samplings were performed on flowstone substrate, with comparable structure and mineral composition (secondary microcrystalline calcite). Concerning diatoms, we assumed that silicate is unlikely to be below limiting concentrations, as pointed out by Round et al. (1990). Caves are undoubtedly oligotrophic environments and we hypothesized a substantial uniformity in nutrient availability in the flowstone walls. Considering the difficulty in sampling seeping water on flowstones for laboratory analysis, we checked nutrient concentrations in a pool which collects seeping water in the central part of the cave (Sala dell'Orso) with the following results: N-NO3: 0,744 mg/l; N-NH4: 0,001 mg/l; SRP: 0,007 mg/l. We collected the data in November 2012. In order to avoid the confounding effect provoked by summer air circulation (Badino 2004, 2010), we chose to sample during the cold season, when the atmosphere in the cave is stable and the air exchange with the external atmosphere is very limited.

Sampling occurred approximately one year after the last cleaning session, thus we assumed biofilms to have reached their growth plateau. Indeed, we aimed at working on mature biofilms to avoid confounding effects caused by different colonization phases (Mulec et al. 2008).

Biotic variables

In each sampling point we measured three replicates of chlorophyll *a* (hereinafter chl-*a*) density (proxy of primary production) of cyanobacteria, green algae and diatoms with BenthoTorch®, developed by BBE Moldaenke GmbH (Schwentinental, Germany). BenthoTorch® is a Pulse Amplitude Modulated (PAM) fluorimeter emitting light pulses at three different wavelengths (470, 525 and 610 nm) within the range of the specific excitation spectrum of the chlorophyll fluorescence. The pigment response to the light pulses is recorded at 690 nm wavelength. Thanks to an inbuilt algorithm, BenthoTorch® calculates an instantaneous and *in situ* measure of the chl-*a* density of the three target benthic photosynthetic organisms composing the biofilm (µg chl-*a*/cm^2). The instrument discriminates the three groups on the base of the different pigments distinctive of the target groups (phycocyanin for cyanobacteria, chlorophyll c and xanthophylls for diatoms and chlorophyll b for green algae). A 700 nm LED is used to compensate the reflectance of the background (Carpentier et al. 2013). It must be emphasized that, according to Kahlert & McKie (2014), measurements obtained with the BenthoTorch® should not be intended as an indirect measure of algal biomass and biovolume, but only as a proxy of biofilm primary production. The use of BenthoTorch® is growing for *in situ* bioassessment in river ecology (see Kahlert and McKie 2014 and citations therein) while it has never been applied to monitor photosynthetic biofilms in caves until now.

Considering that we were interested in examining which environmental variables maximize photosynthetic activity, we used the maximum value measured at each of the three replicates for each sampling point for each photosynthetic group.

Environmental parameters

According to Albertano (2012) and Falasco et al. (2014) we identified light, substrate moisture, temperature, tourists, proximity to external sources of autotrophic organisms and presence of flowing water as the potential factors affecting the presence and the primary production of photosynthetic biofilms on flowstone. For this reason, we chose the following environmental parameters as proxies for the above-mentioned factors and measured them at each plot: illuminance (lux), photosynthetically active radiation (PAR, µmol photons/m^2s) and distance from light sources (m) as proxies for artificial lights; maximum, minimum, mean, range and coefficient of variation of temperature (°C) as proxies for temperature; presence/absence of seeping water as proxy for substrate moisture; distance from the touristic path (m) as proxy for direct tourist impact; distance from the cave entrance (m) as proxy for the proximity to external sources of autotrophic organisms; and distance from the subterranean river (m) as proxy for the presence of flowing water.

Photosynthetically active radiation (PAR) was detected with a DELTA OHM S.r.l. DO 9721 probe and illuminance with a DELTA OHM S.r.l. LP 471 PHOT probe. The smallest distance from the light source and from the touristic pathway were measured in the field by means of a measuring tape, while the distance from the entrance and the distance from the subterranean river were measured in GIS environment on the planimetrical drawing by Capello (1954).

At each sampling point we measured local temperature by means of i-buttons (Hygrochron™ devices), with a measurement range from -20.0 °C to 85.0 °C (±0.5 °C). Dataloggers were placed at each sampling point (for a total of 123 dataloggers) for two weeks during the sampling period, and set to one measure per hour. Data obtained from the dataloggers were used to generate mean, maximum, minimum, range and coefficient of variation.

We also measured air humidity, but the high and almost constant values of air saturation typical of cave environments prevented dataloggers from collecting correct data of relative humidity. We evaluated moisture at each sampling point by recording presence/absence of seeping water on the substrate.

Data analyses

We firstly explored data (see Appendix A) in accordance with Zuur et al. (2009, 2010). We used Cleveland dotplots and boxplots to assess the presence of outliers and avoid unusual observations to exert an undue influence on estimated parameters (Zuur et al. 2009). We then evaluated multicollinearity among predictors using Pearson correlation test and variance inflation factors (VIFs). Variables highly correlated (R^2 correlation value >0.05 and VIF > 2) were excluded to avoid confounding effects and model overfitting (Zuur et al. 2009). Multi-panel scatter plots were used to investigate non-linear relationships between variables, which cannot be detected via correlation tests. In accordance with the results obtained from these analyses, we selected the covariates that were more strongly correlated with our dependent variables. We thus chose seven predictor variables, namely: illuminance, presence of seeping water, maximum temperature, distance from the entrance, distance from the subterranean river and distance from the touristic pathway. A log-transformation was applied to illuminance to achieve homogenization of its distribution, in accordance with Zuur et al. (2009).

We tested the predictor variables and potential interactions against the dependent variables via generalized linear mixed models (GLMMs, in accordance with Zuur et al. 2009) in R environment (R Core Team 2013). Given the spatial dependence of the data (three points on each sampling plots at different distance from the light source), we applied the mixed procedure to include a grouping variable (plot) as a random factor in order to account for the variation it introduced in our samples, rather than to test for its direct effect on the dependent variables.

Considering that our dependent variables could not assume negative values, we discarded the approach of linear regression (since Gaussian distribution allows prediction of negative values). Given the presence of zero values in the dependent value, we performed Zero-Inflated Gamma (ZIG) models, as suggested by Mills (2013). According to the ZIG approach we run two separate models, namely a binomial-GLMM and a gamma-GLMM. We used the former to test the probability that an outcome is a non-zero value and we used the latter to deal with strictly continuous non-zero values. As a consequence, the ZIG approach provides two different outputs: in our case the first

output explains which covariates are involved in the colonization of flowstone by each of our target groups, while the second explains their role in determining values of chl-a density.

We run the binomial model using a complementary log-log link function (*clog-log*), as recommended in Zuur et al. (2009) for datasets with unbalanced set of zeros (absences) and ones (presences). For the gamma models we used a *log* link function. GLMM were fitted via the lme4 R package (Bates et al. 2014, version 1.0-6).

In order to identify the best hypothesis supported by observations, we applied model selection (Johnson & Omland 2004) (see Appendix B). We performed a backward elimination, progressively excluding variables according to AICc values (Zuur et al. 2009). Variables not contributing to the fit of the model (i.e. variables increasing the AICc value) were progressively dropped from the models thus avoiding overfitting (Howkins 2004). We preferred the AICc over the AIC because of the small dimensions of datasets (Hurvich & Tsai 1989).

In order to investigate the existence of possible non-linear responses, we applied generalized additive mixed models (GAMM) that allowed us to fit a non-linear effect (smooth) of the covariates on the dependent variables. We fitted these models via the mgcv R package (Wood 2011, version 1.7-27), taking into account the structure identified according to the previous model selection procedure. Non-linear trends were rejected by plotting the smooth of the covariates against the observations. These plots revealed clear linear patterns in all cases and confirmed the validity of the estimate values generated by GLMM.

GIS processing

The raster image of the planimetric drawings by Capello (1954) was projected “on the fly” in GIS environment on which we drew the vector layer of the Bossea show cave. Areas characterized by the presence of non-flowstone substrates were added on the map on the base of field observations.

For the interpolation we used an Inverse Distance Weighted function (IDW) using a sample of 12 plots (power 3) to estimate cell values.

We used the same procedure to obtain the renderings of the dependent variables (chl-*a* density of cyanobacteria, green algae and diatoms) and illuminance. In order to obtain a more realistic representation we included a number of additional observations for illuminance (90 plots) and the dependent variables (52 plots).

3. Results

Temperature measured at the cave entrance and at the deeper part of the cave were similar, thus attesting the thermic stability of the cave (Badino 2004, 2010) and the consequent general atmosphere stability inside the cave during the sampling period (see Fig. 2).

The average temperature recorded by the dataloggers during the sample period (two weeks) at the sampling points was 8.94 (± 0.34) °C, ranging from 8.14 °C to 18.5 °C. The lowest values were recorded next to the subterranean river. Illuminance in the Bossea show cave ranged from 0.43 to $3.07*10^3$ lux, with an average value of $2.87*10^2$ ($\pm 5.95*10^2$) lux.

Chlorophyll-*a* concentration ranged from 0.00 to 5.90 µg/cm^2 for cyanobacteria, from 0.00 to 6.14 µg/cm^2 for diatoms and from 0.00 to 4.32 µg/cm^2 for green algae. Actually, we recorded the highest values for cyanobacteria (15.20 µg/cm^2) and diatoms density ($3.22*10^2$ µg/cm^2) on the Monache wall, located almost at the end of the touristic pathway. Because of their high values, these points were considered outliers and thus not included in the final dataset used for the statistical models in order to avoid confounding effects (see Appendix A). When outliers were excluded, the mean density values were 0.45 (±0.87) µg/cm^2 for cyanobacteria, 0.55 (±0.94) µg/cm^2 for diatoms and 0.29 (±0.69) µg/cm^2 for green algae.

Statistical models

Significant variables included in the models after model selection are reported in Table 1 (binomial-GLMMs) and Table 2 (gamma-GLMMs).

Cyanobacteria

As long as cyanobacteria are considered, the final binomial model (presence/absence data) uniquely included illuminance, which proved to have a highly positive effect (see Fig. 3a). For chl-*a* density (gamma model) we used 110 observations, obtained after eliminating outliers and zero values. The best-fit model involved illuminance (highly positive significant effect) and presence of seeping water (positive significant), with higher densities on illuminated substrates with seeping water (see Fig. 3b).

Diatoms

Results obtained from the binomial model show a positive effect of illuminance. The effect of seeping water also proves to be significant, with higher suitability of moist substrates (see Fig. 4a).

We fitted diatom chl-*a* density models (gamma) on 110 observations, obtained after eliminating outliers and zero values. The best-fit model describing diatom chl-*a* density included a positive effect of illuminance (see Fig. 4b).

Green algae

The best-fit binomial model for green algae included a significant positive effect of illuminance. A negative effect of seeping water was also significant, with dry surfaces being more suitable to green algae colonization (see Fig. 5a).

Green algae chl-*a* density models were fitted on 76 observations, obtained excluding outliers and zero values. In the final model, the most significant variable was illuminance, demonstrating a positive effect of light intensity. The presence of seeping water proved to influence negatively green algae densities, with higher values on points without seeping water (Fig. 5b). We also observed a significant negative effect of the distance from the entrance (Fig. 5c), thus indicating a higher primary production of green algae in the nearby of the cave entrance than in deeper parts.

Observed values of illuminance and dependent variables (chl-*a* of cyanobacteria, diatoms and green algae) were interpolated in order to produce the maps presented in Fig. 6 and 7 respectively.

4. Discussion

Lampenflora represents a major threat for show caves, where it heavily proliferates in consequence of the installation of artificial lights. In particular, cyanobacteria, diatoms and green algae are the three main photosynthetic groups responsible of the formation of patinas causing aesthetic and structural damage to cave speleothems (Aley 2004). Moreover, the growth of photosynthetic biofilms is also responsible of the chemical alteration of rock surfaces (Albertano et al. 2003).

The understanding of the environmental parameters driving the growth of autotrophic biofilms is a first step towards the correct management of show caves. This study suggests that both colonization and the primary production of photobiota is ruled by complex interacting factors, among which illuminance appears to be the main driver.

In particular, we were able to discriminate among the main groups composing autotrophic patinas in the study area with BenthoTorch®, which we used in the cave environment for the first time. The use of BenthoTorch® allows a rapid assessment of the composition of the biofilm, both in terms of presence and density, providing a precise measure of three suitable indicators for human impact on caves. Such a quantitative characterization is fundamental in order to evaluate the factors influencing the colonization and the growth autotrophic biofilms in caves.

It is widely recognized that cyanobacteria, diatoms and green algae are the most representative groups of photobiota in subterranean environments (Dayner & Johansen 1991; Hoffmann 2002; Smith & Olson 2007; Mulec 2012). When caves are equipped with artificial light systems, green algae are the first colonizers (Mulec et al. 2008). However, green algae communities in caves are generally dominated by species which presumably could not survive in the dark for long periods (Hoffmann 2002). For this reason, at mature stages, photosynthetic biofilms become more similar to those found in the nearby of cave entrances, which are generally dominated by cyanobacteria, overgrowing algae and progressively dominating the biofilms (Mulec et al. 2008; Mulec 2012). Even if diatoms are generally not dominant in cave patinas (Hoffmann 2002; Mulec et al. 2008;

Roldán & Hernández-Mariné 2009), they may become particularly abundant in sites with seeping water (Mulec 2012).

In our study, cyanobacteria occurred in almost all sampling points and showed the highest productivity among the targeted groups. Diatoms were also frequent, with comparable, slightly lower chl-*a* density values. On the contrary, green algae were rare and their chl-*a* density was usually lower. In general terms, according to Mulec et al. (2008), the benthic biofilms observed in Bossea are typically mature, coherently with our hypothesis.

Maximum values of chl-*a* were generally higher than those found in other studies (Mulec et al. 2008). However, measurements were performed with traditional methods adopted for limnological studies (e.g. Vollenweider et al. 1974) and not specifically conceived for biofilms.

In general, the mean value of total chl-*a* that we obtained with BenthoTorch® is similar to the values observed in oligotrophic aquatic habitats, namely artic-alpine, boreal and headwater streams (Kahlert & McKie 2014). From an ecological point of view, we can assert that artificial lights sustain a significant primary production, driving a strictly heterotrophic ecosystem to autotrophy, with significant consequences on higher trophic levels. As pointed out by Mulec et al. (2008) this additional energy input can indirectly affect cave fauna with negative impacts on the most specialized organisms.

As largely expected, illuminance positively influences the presence and the growth of our autotrophic target groups. Because of their strong correlation, the effect of illuminance could also be referred to the photosynthetic active radiation (PAR) that we excluded from the model for avoiding multicollinearity among covariates.

As long as microorganism growth is considered, similar results were reported by Mulec & Kosi (2009), Roldan & Hernandez-Mariné (2009) and Lamprinou et al. (2012), who observed thicker biofilms at higher illuminances. This relation is also consistent with Borderie et al. (2014), who observed that longer periods of illumination appeared to be responsible for greater photoautotrophic biofilm growth in a show cave.

The strong response to illuminance could also be due to the presence of halogen lamps in Bossea cave, which mostly favour the growth of *lampenflora* according to Mulec (2014). Indeed, the chlorophyll-a energy absorption efficiency is particularly favoured within the light spectrum of halogen lamps, as shown in Fig. 1b.

Among the three groups, cyanobacteria showed the most significant response to illuminance, even if several authors demonstrated that an excessive intensity of light could potentially limit their growth (Albertano et al. 2005; Mulec et al. 2008). For this reason, we hypothesized a non-linear response to light, with a peak at intermediate values and a possible decrease at higher intensities. However, we statistically rejected this hypothesis, confirming at least in our range of values, a linear response pattern of cyanobacteria versus illuminance.

The presence of seeping water on the substrate proved to be an important factor driving the both the colonization and the primary production of cyanobacteria and diatoms. In particular, in accordance with the results from the binomial model, seeping water resulted a determinant factor for the colonization of diatoms. This result is coherent with other works, which highlighted the importance of moisture for diatom survival (St. Clair & Rushforth 1976; St.Clair et al. 1981; Padisak et al. 1984; Dayner & Johansen 1991). According to literature, diatom growth seems not to be favoured on carbonatic substrates (St. Clair et al., 1981), but the presence of seeping water may contribute to the diatom establishment because it reacts with limestone releasing Ca^{2+} ions, which are needed by diatoms to adhere to rock surface (Mulec 2012). Results concerning environmental preferences of diatoms are also consistent with previous data collected in the Bossea show cave (Falasco et al. 2015). In particular, in this paper, light and humidity were pointed out as important variables in determining the autoecology of the diatom species dominating the assemblage.

As long as cyanobacteria are considered, the positive effect of the presence of seeping water could be a mirror of its role in transporting microorganisms inside the cave (Mulec et al. 2008). From this point of view, cyanobacteria as well as diatoms probably enter the cave with the water seeping through the rock fractures and reaching the illuminated substrates.

On the contrary, according to our results both presence and chl-*a* density of green algae is favoured on relatively dry surfaces. A similar trend was highlighted by Cennamo et al. (2012), who observed that in roman catacombs green algae were found mainly in dry sites.

It should be pointed out that flowstones in caves are always humid because of the atmosphere saturation. The main difference in terms of surface moisture are due to the presence or absence of seeping water on their surfaces.

Considering that green algae usually represent the first colonizers of the biofilm (Mulec et al. 2008), we can hypothesize that their survival and growth on moist substrates is limited by the competition with cyanobacteria and diatoms. While diatoms and cyanobacteria share common strategies of colonization via the water seeping on the cave walls, green algae seems to have a different strategy. The trend highlighted here points out a positive effect of the proximity to the cave entrance, for which we may hypothesize that green algae are carried into the cave by other factor than seeping water, such as, for instance air or other ways of transport. Our hypothesis seems in accordance with Mulec (2012), who underlines the importance of local air circulation in disseminating propagules of photosynthetic organisms.

According to our results, neither the proximity to the tourist pathway nor to the subterranean river has any effect on our target groups. We therefore assume that allogenic inputs of propagules due to tourists or the presence of flowing water due to the subterranean river are not involved in the colonization or growth of autotrophic biofilm.

The interpolated surfaces of the chl-*a* density of the three autotrophic groups show scarce correspondence among each other, especially between green algae and the other two groups, strengthening our hypothesis of exclusive competition. Despite some overlap, in general they show different patterns of density within the cave. Green algae are more abundant in the proximity of the entrance, while cyanobacteria and diatoms are more spread along the cave, with the highest concentrations next to the light sources. Particular attention should be focused on the Monache wall, at the bottom right of Sala Garelli. This wall is characterized by potentially unfavourable environmental conditions to autotrophic biofilms growth (low illuminance). However, the

concentration of diatoms and cyanobacteria is the highest measured in Bossea show cave. We suppose that this area has been rarely cleaned due to the difficult access, thus allowing diatoms and cyanobacteria to grow undisturbedly through time. This phenomenon highlights the importance of regularly removing microorganisms propagules in all areas open to the public.

Suggestions for management

From the management point of view, our work suggests that particular attention should be focused on illuminance, in order to reduce the growth of autotrophic biofilms on speleothems. More attention should be devoted to implement lightning systems and regimes which are unfavourable for the growth of photosynthetic microorganisms. For instance in accordance with our results, halogen lights should be replaced with lights with lower intensity (Mulec 2014) and their distance from the speleothems should be increased in order to reduce direct illuminance (Cigna 2011). Lights could be provided of a motion sensor, at least along the touristic pathway, in order to reduce the time of light exposure (Grobbelaar 2000). Halogen lamps bulbs could also be replaced with coloured lights with a spectrum out of the absorption peak of chl-*a*, like green and yellow lights (Grobbelaar 2000; Albertano et al. 2005; Roldán et al. 2006; Mulec 2012) because eukaryotic algae have difficulties in adsorbing these wavelengths. On the other hand, cyanobacteria can exploit even green and yellow lights, so this solution would not be effective for their removal (Albertano et al. 2005), but it would possibly reduce their growth (Roldán et al. 2006). An innovative solution would be the installation of LED lightning systems, since LED lights are less favourable to microalgae growth (Mulec, 2014). Moreover, LED lights have the potential of tuning the desirable emission spectrum.

Measuring the chl-*a* density with BenthoTorch® could help in detecting the most damaged areas where supplementary cleaning sessions should be scheduled. Indeed, chemicals generally used for removing biofilms are toxic for cave fauna and tourists (Mulec & Kosi 2009) or may damage cave speleothems (Faimon et al. 2003). Their use should be therefore limited to the minimum necessary to contain biofilm growth.

Considering that Bossea show cave is also exploited for social events, in which lights are turned on for long periods, reasonably recovery periods should be planned, as suggested by Falasco et al. (2014).

5. Conclusions

In our paper, we explored the main environmental drivers determining photobiota productivity in the Bossea show cave. Illuminance resulted an important factor in determining both presence and growth of cyanobacteria, diatoms and green algae, however the three groups exhibited a different response to other factors such as moisture and distance from the cave entrance. As a consequence, the most vulnerable areas in the show cave are not necessarily the most illuminated, but the one subjected to specific combinations of environmental factors.

Our results could be reasonably extended to other show caves with similar environmental features and touristic exploitation. In particular, it would be worth to apply the same methodology in other caves to compare the results and highlight differences. In a second phase, the same statistical approach could be extended to a broader scale in order to evaluate which macro-environmental features are responsible for the growth autotrophic microorganisms in other show caves. In this perspective the effect of other environmental factors such as the number of visitors (Cigna 1993), the duration of the light period (Aley 2004), the type of substrate (Mulec et al. 2008), the size of the cave entrance (Lauriol et al. 2006), CO_2 concentration (Mulec 2012; Aley 2004; Hoffmann 2002), the type of lights employed (Mulec 2014) and type of cleaning practices carried on in the cave (Faimon et al. 2003) should also be considered.

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Figure captions

Figure 1. Light spectrum of halogen lamps in comparison with the absorption peaks of chlorophyll-a (a) and absorption efficiency of chlorophyll-a exposed to halogen lamps with an absorption peak at 660 nm (b).

Figure 2. Daily mean temperature at the Bossea show cave from August 2012 to April 2013 at the cave entrance (dotted line) and 2 km inside the cave (continuous line). During the sampling period (straight bold line), the temperature outside and inside the cave are similar, thus guaranteeing the atmosphere stability inside the cave.

Figure 3. Predicted values (black continuous line) and confidence intervals (95%, light grey area) for cyanobacteria probability of presence (a) and density (b) in relation to illuminance. Trends for absence (left) and presence (right) of seeping water are illustrated.

Figure 4. Predicted values (black continuous line) and confidence intervals (95%, light grey area) for diatom probability of presence in relation to illuminance (a) and density in relation to illuminance (b). Trends for absence (left) and presence (right) of seeping water are illustrated.

Figure 5. Predicted values (black continuous line) and confidence intervals (95%, light grey area) for probability of presence of green algae in relation to illuminance (a) and green algae density in relation to illuminance (b) and distance from the entrance (c). Trends in absence (left) and presence (right) of seeping water are illustrated.

Figure 6. Interpolated surface of illuminance (Lux) inside the Bossea show cave with indications of the main cave attractions.

Figure 7. Interpolated surface of density ($\mu g/cm^2$) of cyanobacteria (a), diatoms (b) and green algae (c) in the Bossea show cave.