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# *In situ* photosynthetic yields of cave photoautotrophic biofilms using two different Pulse Amplitude Modulated fluorometers

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ABSTRACT

In order to evaluate the photosynthetic efficiency of cave photoautotrophic biofilms, *in vivo* chlorophyll *a* fluorescence was measured using two different fluorometers, in two biofilms in the touristic karstic Nerja Cave (Spain). The study was done for entire days in summer and in winter during the first year and repeated for a second year, in order to cover the widest range of environmental conditions, *i.e.*, atmospheric $CO_2$, temperature, seeping water and relative humidity levels. Effective quantum yield and relative electron transport rate (rETR) were determined during periods of light whereas maximal quantum yield ($F_v/F_m$) was determined *in situ* during dark periods. On summer days, *in situ* photosynthetic yields in cyanobacterium biofilms (*Chroococcidiopsis* sp.) increased 7–16 times compared to that of winter days, whereas in biofilms comprised of green and red microalgae and various cyanobacterium species, no seasonal or yearly variations were observed. In contrast, maximal rETR in the two biofilms increased in the periods with the highest values of both $CO_2$ and relative humidity. Positive correlations between all environmental variables and rETR were found. According to Redundancy Analysis, all environmental variables, mainly $CO_2$ and relative humidity were related to photosynthetic variables. The effective quantum yields showed different values depending on the measuring light of the PAM. The values were higher with red light (Diving PAM) compared to blue light (Junior PAM) mainly in the site dominated by cyanobacteria. Nerja Cave is shown as an excellent place to study the effects of light and $CO_2$, among other environmental variables of biofilm photosynthetic activity. The monitoring of photosynthetic activity by *in vivo* chlorophyll *a* fluorescence could be used to follow the effects of the treatments applied by the touristic cave managers to reduce the proliferation of biofilms composed of various species, and, consequently, the biodeterioration of speleothems could be reduced.



## 1. Introduction

Autotrophic biofilms can grow in different environments, such as the soil of deserts, coastal intertidal systems, estuaries, in sediments of deep waters, and in the entrance caves, among others [1–4]. Biofilms can also proliferate on speleothems inside of touristic or "show caves" since they are illuminated by artificial light and they present favorable conditions for photosynthetic activity due to the high humidity, relative constant temperature, and high levels of $CO_2$ [3–6]. Biofilms alter the natural color of the stones, producing the biodeterioration of speleothems [7–9], *i.e.* "maladie verte" [10] or "lampenflora" [11]. There is high interest in avoiding or decreasing the biofilm cover in tourist caves by using physical, mechanical, and chemical methods [12]. One strategy is to apply light/dark treatments to reduce the photosynthetic activity and consequently the biomass proliferation. For example, photosynthetic activity can be decreased by increasing the dark period during the cave's visiting hours, the use of weaker-intensity lamps, the application of LEDs with light qualities enriched in the wavelength with less photosynthetic quantum efficiency for microalgae and cyanobacteria, and, finally, the application of UVC radiation ($\lambda = 200$–280 nm) in order to damage the biofilms [12,13]. This research field is a great opportunity for photobiologists to conduct *in situ* studies on the photophysiology of the biofilms under different environmental conditions and evaluate the bio-optical procedures done to eradicate, reduce, or control the biofilm growth. Generally, in touristic caves, physical and chemical environmental conditions are monitored and thus large-scale tests of the relation between biofilms physiology and environmental variables can be conducted.

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