



The effect of light regimes on a picoplanktonic community from El Quisco Bay, Chile

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Light is an essential source of energy for life on Earth and one of the most important signals for organisms to obtain information from the environment. About 50% of net primary production occur in the ocean, mostly by planktonic photosynthetic organisms that only account for approx. 1% of global primary biomass. In productive coastal waters, where phytoplankton dominates biomass and productivity, picoplankton eukaryotes (PPE < 3µm diameter) represent an important carbon source for the heterotrophic protists. These grazes on the small size fractions and are most abundant in oligotrophic waters. The aim of this work was evaluate the effect of light regimes on the photosynthetic picoplankton microorganisms in mesocosm experiments. Light regimes influence the abundance of different planktonic populations. Photosynthetic eukariotic picoplankton tends to be more sensitive to decrease in light intensity than *Synechococcus*, other bacteria are not affected by light intensities. Interestingly, the maximum fluorescence occurs at 10% of the incident light intensity; it was not correlated with the abundance of the different populations at this light intensity.

INTRODUCTION

Both, eukaryotic and prokaryotic phytoplankton organisms colonize the upper part of the water column, down to the limit of light penetration. Structure and abundance of organisms are controlled by nutrient availability and light. Normally, the phytoplankton displays a fairly predictive annual cycle, however, at small time scales, phytoplankton growth and cellular division is tightly coupled with the diurnal light cycle (Vault et al., 2008). In clear ocean water, visible light decreases approximately 10-fold for every 75 m, which means that at 75 m the light is 10% as bright as it was at the surface; and at just twice that depth, there is only 1% of surface light left.

Confined seawater, in micro/mesocosms, is often used to assess the dynamics and metabolic rates of microbial plankton communities. In such systems incubation times up to 24 h are frequently used to measure community respiration rates and bacterial and primary production. The key assumption of experiments with partially enclosed incubations is that the measured rates adequately represent the one in nature, thus allowing their extrapolation to in situ communities (Calvo-Díaz et al., 2011)

Microcosms were developed and the phytoplankton was characterized with respect to light regimes in confined water. We measured changes in auto- and heterotrophic picoplankton biomass during 96-h of incubation in different regimes of light. An attempt was made to reproduce the light stratification found in the ocean water column. The measurements focused on following *Synechococcus* sp., total bacteria and photosynthetic picoeukaryotes. We obtained high quality data for cell counts, cell by cell fluorescence by flow cytometry and in vivo total chlorophyll fluorescence.

Light regimes influence the abundance of different planktonic populations. Photosynthetic eukaryotic picoplankton tends to be more sensitive to decrease in light intensity than *Synechococcus* sp. in mesocosms, the total number of bacteria is not affected by light intensities, which is in accordance with the vertical distribution. Interestingly, the maximum fluorescence occurred at 10% light intensity that was not correlate with the abundance of the different populations at this light intensity.

METHODOLOGY

Sampling: Incubation experiments with natural phytoplankton assemblages took place in El Quisco Bay. (33°23'21.69"S, 71°44'51.44"W). For microbiological analysis 4 different depths from the water column were sampled, and the 5m depth was chosen for the microcosm experiment. Samples were taken using a 5 l Niskin bottle. Samples were collected in duplicate, using 5 l carboys (previously washed with 1% HCl) for storage. The samples were then pre-filtered with a net of 150 µm pore size, in order to exclude higher organisms and particulate material.

Water column physicochemical parameters were determined with a conductivity, temperature and depth (CTD) monitor as far as 80 m deep.

Flow cytometry 1.5 ml water sample aliquotes were fixed with 0.1% glutaraldehyde (final concentration), frozen in liquid nitrogen and stored at -80°C until analysis. Flow cytometry analysis was performed based on the side scatter red chlorophyll fluorescence and orange phycoerythrin fluorescence (for *Synechococcus* detection).

Microcosm experiment: Incubations were done in clear polyethylene 10-L containers. Screening nets were used to lower the sunlight intensity reaching the inside of the containers; 99 % light intensity attenuation yielded maximum irradiance of 1 µmol photons /m² x sec; 90% light intensity attenuation yielded maximum irradiance of 33 µmol photons /m² x sec. Direct light yielded maximum irradiance of 230 µmol photons /m² x sec.

Samples for cytometry were taken at 24 hours intervals and were processed as described. In vivo community chlorophyll was detected with an Aquafuor handheld fluorimeter (488 nm emission, 692 nm LP detection).

Epifluorescence microscopy: 15 mL samples were centrifuged at maximum speed x 15 min. The supernatant was removed and the pellet was resuspended in a small volume. 20 µL of each sample were analyzed as wet mounts employing a Zeiss epifluorescence microscope at 1000X.

Discussion

- CTD profiles show a stratified water column with the chlorophyll maximum widely distributed over the thermocline. *Synechococcus* sp. and PPEs are distributed over the water column with low abundances.
- The higher fluorescence observed at 10% treatment can be the result of accumulation of pigments in this community to overcome the lower light intensity. ("photo acclimation"). It is well known that phytoplankton cells acclimate to depth by increasing their pigment content and by shifting their pigment composition (for example, *Prochlorococcus* increases its chlorophyll b/a ratio since chlorophyll b absorbs optimally blue wavelengths). (Vault, 2006).
- The picophyto eukaryotes (PPE) community seems to be more sensitive to light changes in enclosed experiments, which is consistent with the results found by Calvo-Díaz and colleagues (2011). These authors observed a decrease in picocautotrophic biomass, typically affecting the picoeukaryotes.

RESULTS

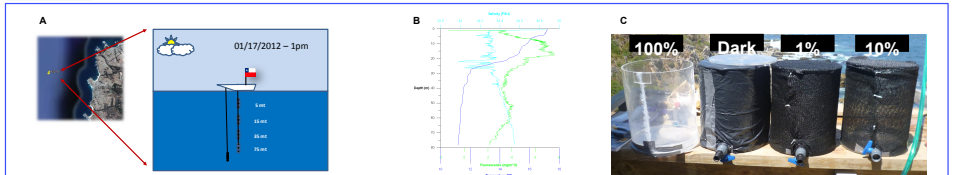


Figure 1. Sampling site, physicochemical properties of the water column and mesocosm experiment design. Samples were taken from four depths (A). CTD data were processed with a seabird program (B). Water from 5 meter depth was used as inoculum for the mesocosms. Experiments were carried out at the ECIM station using four light intensities as described in methods (C).

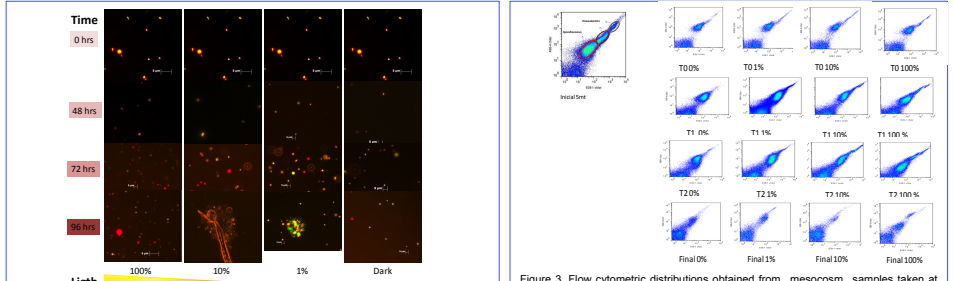


Figure 2. Epifluorescence images obtained from mesocosm samples taken at different light intensities during the experiment. The maximum cell number was observed at 72 hours. Cell aggregation, a proxy for cell lysis, was observed at 96 hours, except at 100% light intensity.

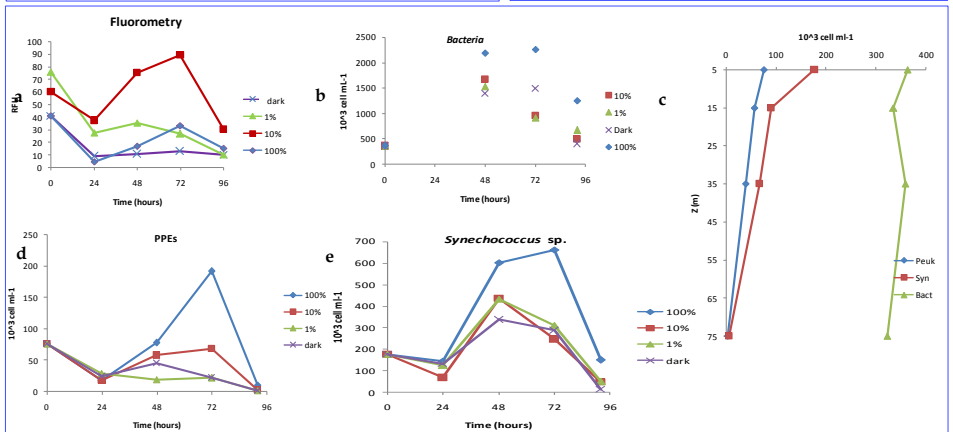


Figure 4. Vertical distribution of mayor picoplanktonic groups at El Quisco Bay (c). In vivo fluorescence & abundance of the mayor picoplanktonic groups in mesocosm experiment. A) In vivo fluorescence expressed as relative units compared with natural seawater from El Quisco. Cell counts for PPEs (d) and *Synechococcus* (e) are based on autofluorescence as described. Total counts of bacteria were determined using Sybr green dye and the 488 nm laser (Sybr detection v/s FSC)

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