Unveiling the Long-Term Effects: A Multigenerational Study on the Impact of Commercial Sunscreens on Marine Microalgae *Phaeodactylum tricornutum*

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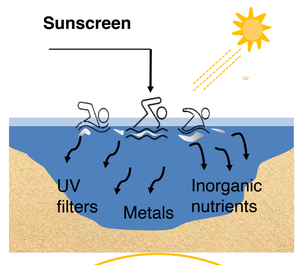
INTRODUCTION

The expanding knowledge about the danger of sunburn, has led to a notorious increase in the use of **SUNSCREENS** around the world. However, these cosmetics are considered **POLLUTANTS** of emerging concern, especially in marine ecosystems. Despite of the number of studies evaluating the detrimental effects caused by the ingredients released from sunscreens on **MARINE ENVIRONMENT**, they have been focused on the short-term responses of a given species, ignoring how marine biota will respond to this new anthropogenic invader when taking into account **TRANS-GENERATIONAL PLASTICITY.**



The main objective of this study is to evaluate the effects of commercial sunscreens in the marine microalgae ***Phaeodactylum tricornutum*** through consecutive generations.

THE WORKING HYPOTHESIS IS THAT SUNSCREENS MAY NOT REPRESENT A DANGER FOR THE DEVELOPMENT OF MICROALGAE AT SHORT BUT ACROSS GENERATIONS.

MATERIAL & METHODS

- A mixture of 5 different **commercial sunscreens** most used in Europe (table 1)
- 5 consecutive **generations.** Each generation lasts 96 hours
- 4 concentrations (**15, 30, 60 and 90 mg.L^{-1}**) and a control (no sunscreen added)
- 4 **replicates** per concentration. **10^4 cells/mL** Initial cell density.
- **Edpoints:** density, metabolic activity, chl-*a and* active chlorophyll fluorescence

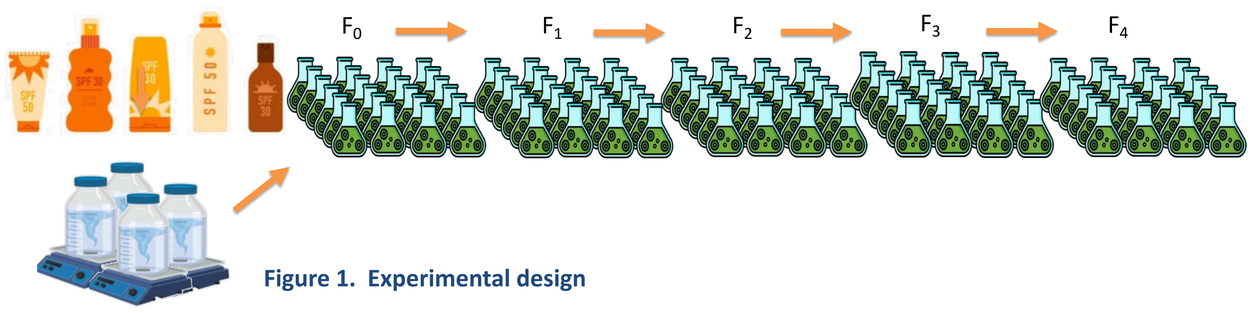


Figure 1. Experimental design

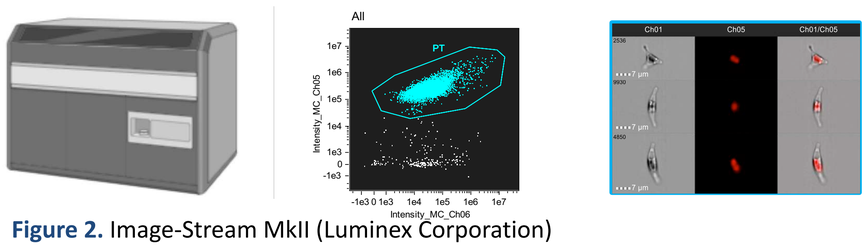


Figure 2. Image-Stream MkII (Luminex Corporation)

- **Cells** were **counted** through Image Flow Cytometry (IFC) (Fig. 2).

Table 1. Characteristics of the three sunscreens selected: SPF (sun protection factor), format (application type) and UV filters included in their formulation.

Sunscreen	Format	SPF	UV Filters[a]
1	spray	50	1, 3, 5, 8, 9, 11
2	gel cream	50	1,2,3, 4, 5
3	gel cream	50	2, 10, 13, 14
4	cream	30	1,3,5,8, 9, 12,13
5	spray	30	2,3,6,7, 8, 9

[a] UV filters: (1) avobenzone, (2) octocrylene, (3) bemotrizinol (4) bisoctrizol, (5) ensulizol, (6) homosolate, (7) Ecamsule, (8) ethylhexyl triazone, (9) octisalate, (10) octinoxate, (11) drometrizole trisiloxane, (12) diethylamino hydroxybenzoyl hexyl benzoate (13) titanium dioxide (nano), (14) zinc oxide (nano)

- For **chlorophyll-a** analysis, pigments were extracted in a 90% acetone solution and measured by spectrophotometer. Data were calculated using the trichromatic equation of Jeffrey and Humphrey (1975)
- **Active chlorophyll fluorescence** was measured fluorometrically using a Phyto-PAM instrument (Heinz Walz GmbH) equipped with an ED-101 US/MP Optical Unit. This parameter measures the efficiency of the photochemical energy conversion process (Schreiber et al., 1995).
- **Metabolic activity** was determined spectrophotometrically using a cell esterase activity assay, through the use of fluoresce in diacetate (FDA) (Jochem, 1999).

RESULTS AND DISCUSSION

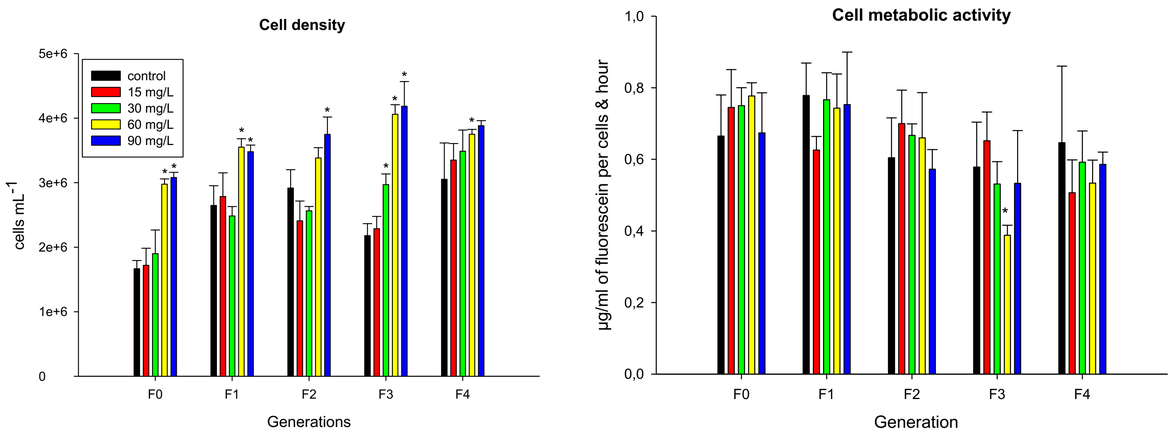


Figure 3. Cell density and cell metabolic activity results.

Significant differences (*p < 0.05) in number of cells (Fig. 3) were found in higher concentrations (30, 60 and 90 mg/L) across all generations, except of F4, compared with the control of each generation.
However, in general, after 96 hours of exposure in each generation, the esterase activity analysis (Fig. 3) revealed that the cell metabolic activity was not significantly affected in any treatment or generation.

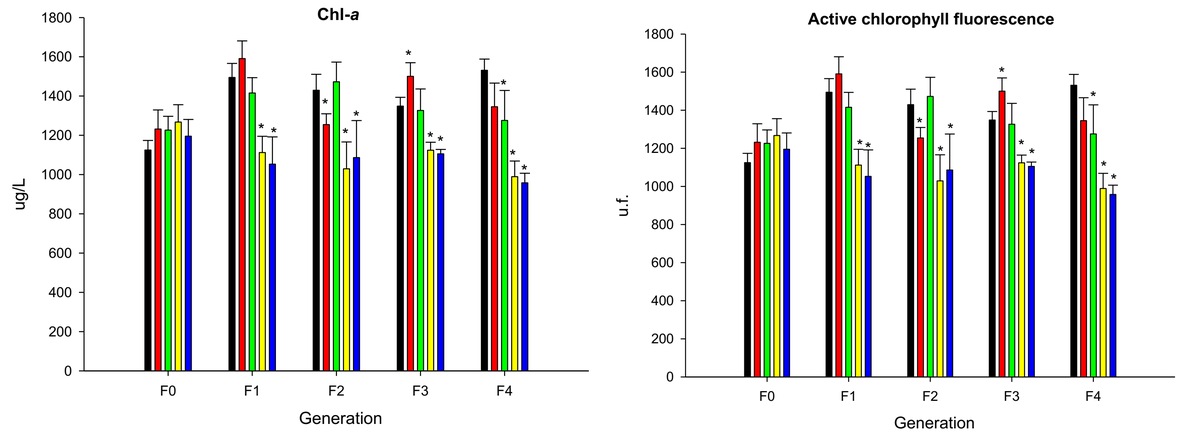


Figure 4. Chlorpphyll-*a* pigments and active chlorophyll fluorescence results.

The concentration of chlorophyll *a* per cell after 96 h through five generations is represented in Figure 4. Statistical analysis showed significant differences (*p < 0.05) between the control and the higher concentrations from F_2 on, with lower concentration of pigments in higher concentrations. Plus, results showed significant differences between the control and the rest of concentration, being highly reduced when high concentrations of sunscreen are added.

CONCLUSIONS

- Results suggest that the release of sunscreens on marine waters may cause **negative effects** on *P. tricornutum* population.
- The damage was measured through the **decrease of pigments** in microalgae exposed to higher concentrations of this pollutant.
- While the number of cells was increased in higher concentrations, probably due to the release of inorganic nutrients from sunscreens, cell metabolic activity was similar, what indicates that **some of the cells may not be functional.**
- The results obtained for the different proposed concentrations of sunscreens are of great importance to understand the potential **knock-on effects** that could happen as a result of the diatom population alterations.

REFERENCES

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