Looking for Biological Monitoring Tools: Using *Anemonia sulcata* as Bioindicator Species to Assess Sunscreen and Ultraviolet Filter in Temperate Seas

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INTRODUCTION

The **conservation of biodiversity and the social and economic drivers of marine ecosystems are directly linked to the identification of environmental stressors**, the application of advanced **marine monitoring techniques** and the contribution to the **value of marine resources**.

Increasing pollution from contaminants of emerging concern, such as **sunscreens**, could accelerate the degradation of marine ecosystems and their resources.

Numerous studies **associate the increased concentration of UV filters with coral bleaching and reef deterioration** in warm areas.

In temperate latitudes, such as the **western Mediterranean**, pollution-related adverse effects such as bleaching of **cnidarians** (corals and anemones) have recently been documented.

In these areas, the symbiotic sea anemone ***Anemonia sulcata*** is gaining **increasing interest as a bioindicator species**, while at the same time the need for monitoring the bleaching phenomenon is increasing.

To assess the rate of adaptation and acclimatization in a changing climate, the **defense mechanism of this potential bioindicator species against the stress of sunscreen pollution will be investigated in the laboratory**.

The identification of potential **biomarkers** of this key species could significantly improve the capacity of **environmental management in temperate marine areas**.

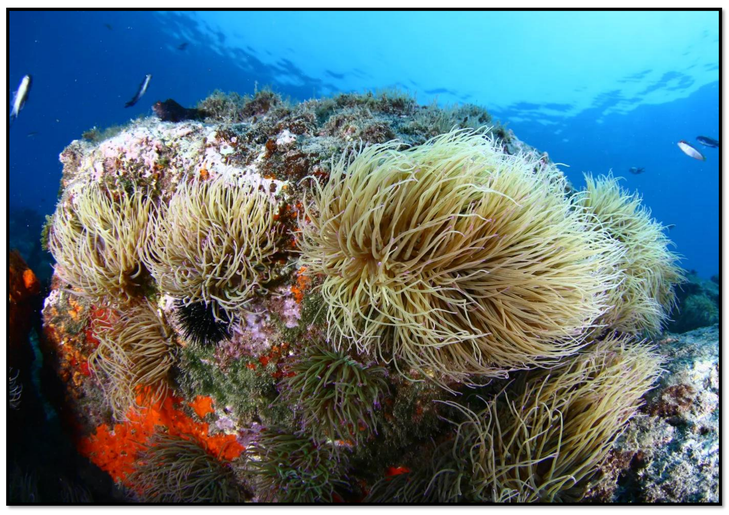


Figure 1. Photo of *Anemonia sulcata* in the sea (https://www.malaga.es)

OBJECTIVE

To **identify and characterize the pollution stress response** to **Benzophenone-3** (BP-3) and a sunscreen containing BP-3, at the individual and cellular levels in the temperate anemone specie *Anemonia sulcata* and its symbiont *Symbiodinium sp.*, in order to **identify biomarkers for monitoring emerging compound pollution in temperate seas**.

METHODOLOGY

Experimental design

Two essays of 7 days

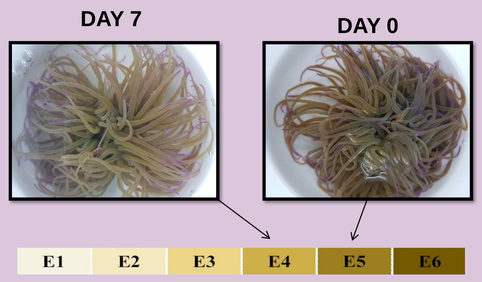
BP-3
- Control
- Negative control (metanol)
- Range from 0,005 to 5000 µg/L of BP-3

Sunscreen contains BP-3 *
- Clean hands control
- 5, 20, 50 and 100% of seawater matrix

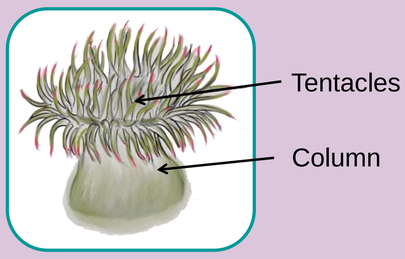
* A seawater matrix was prepared by applying the recommended amount of sunscreen to hands according to the U.S. Food and Drug Administration (2 mg sunscreen/cm²) and immersing them in to 4 liters of seawater for 20 minutes (Danovaro et al., 2008).

Bleaching Measurement

The bleaching of the anemones was measured using a ***Coral health chart*** from the University of Queensland, Australia. (Danovaro et al., 2008). Example of bleaching. Anemone with 500 µg/L treatment of BP-3.



Sampled tissue



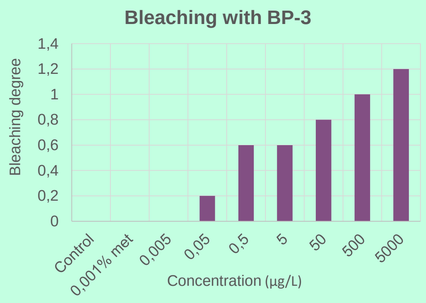
Two grams were cut from both the tentacle and the column of each anemone and analyzed separately.

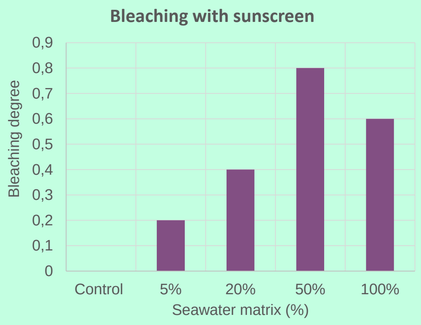
Analytical and stadistical approach

- **Analyltical approach**
  - **Genotoxicity:** DNA damage
  - **Antioxidant defense**
    - Glutathione S-transferase (GST)
    - Glutation reductase (GR)
    - Glutation peroxidase (GPX)
  - **Lipid peroxidation:** LPO
  - Status of *Symbiodinium* sp. cells
- **Stadistical approach**
  - One-factor Anova (Dunnet)
  - Pearson's correlation coefficient

RESULTS

Bleaching Measurement

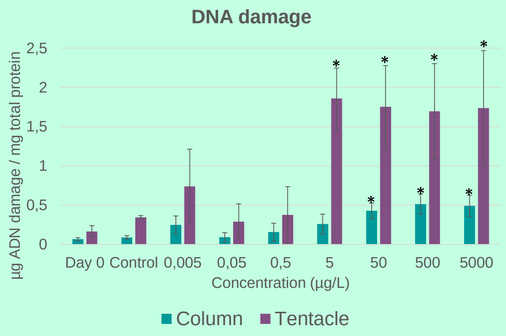


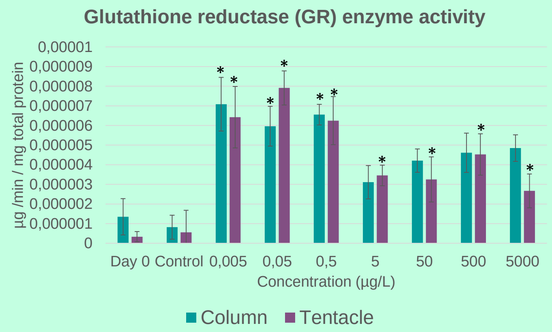


There is a causal relationship between the increase in BP-3 concentration and bleaching ($p < 0,01$).

Biomarkers of oxidative stress

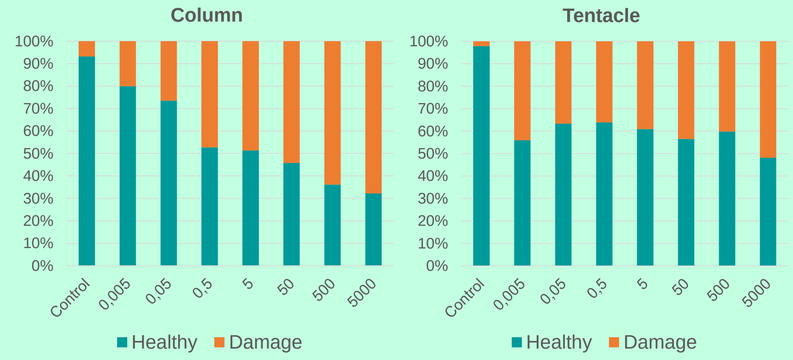
- It's a observed a positive correlation between **DNA damage** and **GR** ($p<0.01$) with **increasing BP-3** concentration.
- Significant differences in **DNA damage** and **GR** biomarkers ($p<0.05$) were determined between the control and **environmental concentrations** for the BP-3 assay.
- Tentacle tissues showed a more sensitive response to increasing BP-3 concentrations than column tissues in all what regards biomarker approach.
- No a clear tendance was observed between increasing percentage of sunscreen and biomarker responses.





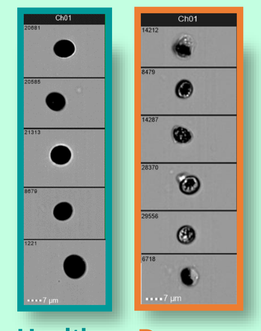
(*) Significant differences with control ($p < 0,05$)

Status of *Symbiodinium* sp. (%)



The percentage of damaged *Symbiodinium* sp. showed that the percentage of damaged cells in the **column** increased with increasing concentration, reaching 61% at the highest concentration.

On the other hand, in the **tentacle,** the percentage of damaged cells remained at around 40% despite the increase in the concentration of BP-3.



Healthy **Damage**

CONCLUSIONS

- ✓ BP-3 is potentially hazardous to marine ecosystems.
- ✓ BP-3 induce oxidative stress and provoke damage in symbiont organisms.
- ✓ BP-3 is an ingredient in sunscreens, and while we are aware of its presence in the environment, the mechanisms and timing of its transition to bioavailability for biota remain unknown, necessitating laboratory experiments.
- ✓ Biomarkers of oxidative stress and genotoxicity have shown to be potential monitoring tools for the assessment of contamination by BP-3.
- ✓ More research should be done regarding time and concentration testing of sunscreens
- ✓ It is crucial to continue advancing the study of these compounds, both individually and within their original matrix, and to standardize protocols while facilitating the transfer of information to the industry.

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