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Valutazione dell'impatto di prodotti per la cura personale sul corallo duro *Seriatopora caliendrum* per identificare soluzioni ecocompatibili

Assessment on the impact of personal care products on hard coral *Seriatopora caliendrum* to identify eco-compatible solutions

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“Sarebbe bello se, per ogni mare che ci
aspetta, ci fosse un fiume, per noi. […]
Una strada da qui al mare.”
A. Baricco, *Oceano mare*

RIASSUNTO

I prodotti per la cura personale sono inquinanti di recente interesse nell'ambiente marino, ed è stato dimostrato che danneggiano una vasta gamma di organismi marini, compresi i coralli duri. Quantità massicce di prodotti per la protezione solare sono rilasciate ogni anno direttamente nelle aree della barriera corallina, e si concentrano principalmente nei siti turistici. Questi fattori di stress chimico potrebbero indurre lo sbiancamento dei coralli a causa della perdita di microalghe simbiotiche, che sono spesso fondamentali per il sostentamento energetico dei coralli. Si è anche scoperto che alcune molecole attivano il ciclo litico dei virus, favorendo le infezioni (Danovaro et al., 2008). Queste informazioni hanno portato alla necessità di sviluppare formule cosmetiche che non contengano composti dannosi per la vita marina. Finora, la maggior parte degli studi si è concentrata sulle risposte degli organismi marini ai filtri UV, sebbene anche altri ingredienti possano risultare dannosi.

Questo studio mira a valutare l'impatto di 13 prodotti di protezione solare, contenenti diversi filtri UV e altri ingredienti, sul corallo duro Seriatopora caliendrum, indagando il loro impatto in termini di rilascio di zooxantelle, arricchimento microbico nell'acqua circostante, e grado di sbiancamento. Abbiamo osservato effetti negativi sul corallo causati da alcuni dei prodotti

testati. In particolare, un grave sbiancamento e un elevato rilascio di zooxantelle sono stati indotti dal trattamento con Hydropuntil.

Questi risultati evidenziano la necessità di testare formule complete o prodotti commercializzati e non singoli ingredienti su organismi marini per definire l'effettiva eco-compatibilità dei filtri solari.

ABSTRACT

Personal care products are emerging pollutants in marine environment, and they have been proven to harm a wide range of marine organisms, including hard corals. Massive amounts of sunscreen products are annually released directly into coral reef areas, mainly concentrating in touristic sites. These chemical stressors could induce coral bleaching due to the loss of symbiotic microalgae, which are often fundamental for the energetic sustainment of the corals. It has also been discovered that some molecules activate the lytic cycle of viruses, promoting infections (Danovaro et al., 2008). This information led to the need to develop cosmetic formulas that do not contain compounds harmful to marine life. So far, most studies are focused on the responses of marine organisms to UV filters although also other ingredients might be harmful.

This study aims to assess the impact of 13 sunscreen products, containing different UV filters and other ingredients, on the scleractinian coral *Seriatopora caliendrum*, investigating their impact in terms of release of zooxanthellae, microbial replication (prokaryotic and viral abundance) in the surrounding water, and degree of bleaching. We have observed negative effects on the coral caused by some of the tested products. In particular, severe bleaching and a

high release of zooxanthellae raised concern on the active ingredient Hydropuntil.

These results highlight the need to test complete formulas or marketed products and not individual ingredients on marine organisms to define the effective eco-compatibility of sunscreens.

TABLE OF CONTENTS

1. INTRODUCTION

Coral reefs are among the most diverse and complex ecosystems on Earth and also among the most heavily exploited habitats by humankind (Miller et al., 2021; Moeller et al, 2021; Downs et al., 2016; Burke et al., 2011; Spalding et al., 2001).

Covering only 250000 km², less than 0.1% of the marine environment, coral reefs support huge biodiversity (around 25% of the marine species, Burke et al., 2011), and provide ecosystem services to half a billion people (Moeller et al., 2021; Miller et al., 2021), including food security (Hughes et al., 2012), financial incomes (Teh et al., 2013) and protection against natural hazards (Ferrario et al., 2014).

Unfortunately, coral reefs are in decline worldwide (Watkins and Sallach, 2021; Wear and Thurber, 2015): approximately 70% of coral reefs are currently threatened by several natural and anthropogenic impacts including overfishing, urban-coastal development, pollution, and tourism (Corinaldesi et al., 2018; Tsui et al., 2017; Spalding and Brown, 2015; Krieger and Chadwick, 2013).

In particular, the excess of ultraviolet radiation (UV), temperature anomalies, the presence of pathogens and pollutants such as sunscreen products

have been reported to be responsible for coral bleaching (Corinaldesi et al., 2018; Hedouin et al., 2016; Danovaro et al., 2008).

The reasons for this environmental degradation are complex, but there is evidence that demographic factors and mass tourism play a significant role (Tovar-Sánchez et al., 2013). It has been also estimated that every year, millions of tourists travel to tropical destinations (Burke et al., 2011; Spalding et al., 2017) with potentially important consequences on environmental contamination (Danovaro et al., 2008). For instance, the Coral Triangle region receives an even greater proportion of people visiting coastal and marine areas than other parts of the world with 33.5 million international visitors only in 2014. Furthermore, in the Caribbean, official estimates indicate that 70000 tons of waste are generated annually from tourism activities (UNWTO, 2015).

Also, the use and release of sunscreens into marine coastal areas are intimately linked to the growth of tourism and awareness of the risks associated with exposure to ultraviolet (UV) radiation (Gilbert et al., 2013; Tovar-Sánchez et al., 2013). Production and consumption of sunscreen products have had a strong growth over the last decades, showing to date the fastest-growing sales globally (Sánchez-Quiles and Tovar-Sánchez, 2015).

This implies a high release of sunscreen products in these marine environments, estimated at 4000-6000 tons/year in reef areas (Danovaro et al.

2008). More recent estimates of sunscreen input into marine environment (Labille et al., 2020) applied to the worldwide number of tourists in coral reefs areas (Spalding et al., 2017), suggest that more than 133700 tons of sunscreen products are released every year in these habitats.

Approximately 25% of the sunscreens applied is not absorbed by the skin and is released into the sea through bathing activity or into the sewage system during the shower (Corinaldesi et al., 2018; Danovaro et al., 2008).

Sunscreen products contain organic (e.g., aminobenzoic acid, ethylhexyl triazone, cinnamates, salicylates, benzophenone, dibenzoyl-methane, benzimidazole) and inorganic filters (e.g., TiO_2 and ZnO), preservatives, adjuvants, moisturizing and antioxidant chemicals.

Several sunscreen ingredients have been detected at concentrations of several hundreds of micrograms per liter in the marine environment (Fagervold et al., 2019; Downs et al., 2016; Tovar-Sánchez et al., 2013; Danovaro et al., 2008; Danovaro and Corinaldesi, 2003).

Due to the lipophilic nature of these cosmetics (Watkins and Sallach, 2021, Danovaro et al., 2008) and the insolubility of some of their compounds (Cadena-Aizaga et al., 2020), sunscreen products tend to bioaccumulate in sediments and aquatic organisms (Huang et al., 2021; Mitchelmore et al., 2019) and could potentially biomagnify in the marine food web (Bachelot et al. 2012;

Gago-Ferrero et al. 2013). The most commonly utilized sunscreens and UV filters, in particular organic filters such as cinnamates, benzophenones, as well as preservatives (i.e., parabens), have been tested for their potential impact on some unicellular and pluricellular organisms (including bacteria, phytoplankton, corals and crustaceans), causing effects similar to those reported for other xenobiotic compounds (Lozano et al., 2020; Thorel et al., 2020; He et al., 2019; Fastelli and Renzi, 2019; Corinaldesi et al., 2018). Furthermore, the properties and persistence of the sunscreens once applied to the skin can be changed due to immersion, UV radiation, temperature, moisture, or abrasion with beach sand (Caloni et al., 2021; Langford and Thomas, 2008; Stokes and Diffey, 2000) leading to the release of altered products into the marine environment.

Organic UV filters are generally more subject to photolysis in comparison to inorganic ones, and this can lead to transformation products, that can negatively affect marine organisms (Watkins and Sallach, 2021). In particular, octinoxate (EHMC), octocrylene (OMC), 4-aminobenzoic acid (PABA) and 2-ethylhexyl 4-(dimethylamino) benzoate (OD-PABA) are most likely to induce reactive oxygen species formation (Caloni et al., 2021).

A recent study (He et al., 2019) compared toxicities of four organic UV filters (benzophenone derivatives) on larvae and adults of two coral species,

Pocillopora damicornis and *Seriatopora caliendrum.* The results showed significant settlement failure, bleaching and mortality on *S. caliendrum* larvae and adults.

Additional studies have revealed that sunscreen ingredients and their active ingredients promote the lytic cycle in marine bacterioplankton (Danovaro and Corinaldesi 2003), and have been shown to cause coral bleaching by promoting viral infections (Danovaro et al. 2008), and to affect coral planulae (Downs et al. 2016) and other reef organisms (Thorel et al., 2020; McCoshum et al. 2016).

These findings had a great impact from a political and economic point of view and led to the ban of chemical sunscreens in several tropical areas and the promotion of sunscreen containing inorganic UV filters (i.e., TiO_2 and ZnO; e.g. Mexico's eco-reserves, Xcaret 2018; Xel-ha 2018). In Hawaii, legislation prohibiting chemical sunscreens containing oxybenzone has already entered into force (Miller et al., 2021; Moeller et al., 2021). However, also "eco-friendly sunscreens", despite they are branded as "natural, biodegradable, reef safe, organic, green", might not be safe for the environment. For example, some plant-based oils found in "organic" sunscreens, such as lavender and neem oil, are also used as insecticides and insect repellents (Maia and Moore 2011; Kanat

and Alma 2004), suggesting potential toxicity for marine organisms, especially arthropods.

Despite the claims of the eco-compatibility of the sunscreen formulations, information on the actual safety to the marine environment is very limited, and in almost all cases the definition of eco-compatibility is not corroborated by rigorous scientific tests on marine life, which ensure the effective eco-compatibility with marine life.

2. OBJECTIVES

This research aims to assess the potential effects of different sunscreen products and active ingredients, containing natural extracts and UV filters previously defined as less impactful.

In particular, the objective of the present investigation is to test the sunscreen products on a tropical hard coral, *Seriatopora caliendrum*, and its symbiotic algae (zooxanthellae).

Seriatopora caliendrum has been selected as a species to be tested due to its higher sensitivity to bleaching in comparison to other hard coral species (He et al., 2019; Zheng et al., 2019; Bhagoolil and Yakovlev, 2004).

3. MATERIALS AND METHODS

3.1 Sunscreen products

We selected 10 different brands of sunscreens and 3 active ingredients with compounds of natural origin (Tab. 1). The names assigned to the sunscreens tested in the experiments are based on the characteristics expressed by manufacturers, and do not correspond to the real name of the products.

Sunscreen products tested contain the following UV filters, in different concentrations: bis-ethylexyloxyphenol methoxyphenyl triazine (BEMT), diethylamino hydroxybenzoyl hexyl benzoate (DHHB), ethylhexyl triazone (EHT), and methylene bis-benzotriazolyl tetramethylbutylphenol (MBBT). The full composition, according to the international nomenclature (INCI), is summarized below, and the Sun Protection Factor (SPF) is also indicated for each sunscreen product.

Suncreen 3 (S3, SPF 30): MBBT 6%, DHHB 4%, EHT 2%, BEMT 1%. Water, adjuvants (e.g., dicaprylyl carbonate), preservatives (e.g., phenoxyethanol), parfum.

Sunscreen 4 (S4, **SPF 30)**: MBBT 6%, DHHB 4%, EHT 2%, BEMT 1%. Water, adjuvants (e.g., dicaprylyl carbonate), preservatives (e.g., phenoxyethanol), parfum.

Sunscreen 5 (S5, **SPF 50)**: DHHB 8%, MBBT 8%, BEMT 3%, EHT 3%. Water, adjuvants (e.g., dicaprylyl carbonate), preservatives (e.g., phenoxyethanol), parfum.

Sunscreen 6 (S6, **SPF 50)**: DHHB 8%, MBBT 8%, BEMT 3%, EHT 3%. Water, adjuvants (e.g., dicaprylyl carbonate), preservatives (e.g., phenoxyethanol).

Sunscreen 7 (S7, **SPF 50)**: DHHB 8%, MBBT 8%, BEMT 3%, EHT 3%. Water, adjuvants (e.g., dicaprylyl carbonate), preservatives (e.g., phenoxyethanol).

Sunscreen 8 (S8, **SPF 50)**: DHHB 8%, MBBT 8%, BEMT 3%, EHT 3%. Water, adjuvants (e.g., dicaprylyl carbonate), preservatives (e.g., phenoxyethanol), parfum.

Sunscreen 15 (S15, **SPF 30)**: MBBT 12%, DHHB 4%, EHT 2%, BEMT 1%. Water, adjuvants (e.g., propylene glycol dicaprylate/dicaprate), preservatives (e.g., phenoxyethanol).

Sunscreen 16 (S16, **SPF 30)**: MBBT 12%, DHHB 4%, EHT 2%, BEMT 1%. Water, adjuvants (e.g., lauroyl lysine), preservatives (e.g., phenoxyethanol).

Sunscreen 17 (S17, **SPF 50+)**: MBBT 16%, DHHB 8%, BEMT 3%, EHT 3%. Water, adjuvants (e.g., lauroyl lysine), preservatives (e.g., phenoxyethanol).

Sunscreen 18 (S18, **SPF 50+)**: MBBT 16%, DHHB 8%, BEMT 3%, EHT 3%. Water, adjuvants (e.g., propylene glycol dicaprylate/dicaprate), preservatives (e.g., phenoxyethanol).

Sensamone P5 (S19, 2%): containing shea (*Vitellaria paradoxa*, Gaertn) butter, maltodextrin, pentapeptide-59, hydrogenated lecithin, water.

Hydropuntil (S20, 3%): containing prickly pear (*Opuntia ficus-indica*, L. Miller) stem extract, glycerin, potassium sorbate, sodium sorbate.

Senseryn (S21, 2%): containing hops (*Humulus lupulus*, Linnaeus 1753) extract, citric acid, propanediol.

3.2 Experimental design

Hard coral fragments belonging to the species *Seriatopora caliendrum* were collected from different donor colonies reproduced and kept in the

aquarium. The coral fragments, which length varied from 3 to 6 centimeters, have been immediately fixed on rigid ceramic support and placed in the aquarium where they were acclimatized for 24 h in conditions of optimal temperature and salinity (26 ° C and 35 psu). After the acclimatization, healthy corals (i.e., with no signs of bleaching or necrotic tissue and with open polyps) were washed in virus-free seawater (filtered on 0.02 μm membranes; Anotop; Whatman, Springfield Mill, UK) and immersed in Whirl-pack polyethylene bags (Nasco, Fort Atkinson, WI, USA) filled with one liter of sea water.

Three replicates were used for each treatment. Corals were exposed to 50μl L^{-1} of different sunscreens and ingredients (Table 1) and compared with untreated systems, used as controls.

For the 3 ingredients SenSamone P5 (S19), Hydropuntil (S20) and Senseryn (S21), the percentages used within the solar formulations were maintained in the experimental system (i.e., 2%, 3% and 2% respectively).

Table 1: List of sunscreen products and ingredients used for the evaluation of eco-compatibility on hard corals.

(*) =% of substance used within the solar formulations

Treatment	Product tipology	SPF/%(*)
S3	Sunscreen	30
S4	Sunscreen	30
S5	Sunscreen	50
S6	Sunscreen	50
S7	Sunscreen	50
S8	Sunscreen	50
S15	Sunscreen	30
S16	Sunscreen	30
S17	Sunscreen	50+
S18	Sunscreen	50+
S19	SenSamone P5	2%
S20	Hydropuntil	3%
S21	Senseryn	2%

3.3 Abundance of zooxanthellae released in seawater surrounding corals

In order to quantify the total number of symbiont microalgae, (i.e. zooxanthellae) released by the coral colonies during the experiment, samples of seawater surrounding the coral fragments were analyzed.

Five mL of seawater were collected from treated systems (Table 1) and the controls, immediately after the addition of the sun products and ingredients (T_0 = beginning of the experiment) and after 18 (T_{18}) and 42 h (T_{42}) from the beginning of the experiment. Aliquots of seawater were filtered on 2.0 µm polycarbonate filters (Nucleopore, polycarbonate, 25 mm diameter, Whatman), which were mounted on slides. Zooxanthellae were observed and counted under the Zeiss Axioplan epifluorescence microscope (Carl Zeiss Inc., Jena, Germany; ×400 and ×1000).

Different impact levels were established when in the treatments the abundance of zooxanthellae at T_{18} or T_{42} was statistically higher than in the control, as follows: slight impact (ratio of zooxanthellae abundances in the treated and in the control systems ≤1.5); moderate impact (ratio between abundances of zooxanthellae in the treated and in the control systems between 1.6 and 2.5); strong impact (ratio between the abundance of zooxanthellae in the treated and in the control systems between 2.6 and 4.5) and severe impact (ratio between the abundance of zooxanthellae in the treated and in the control systems ≥ 4.6).

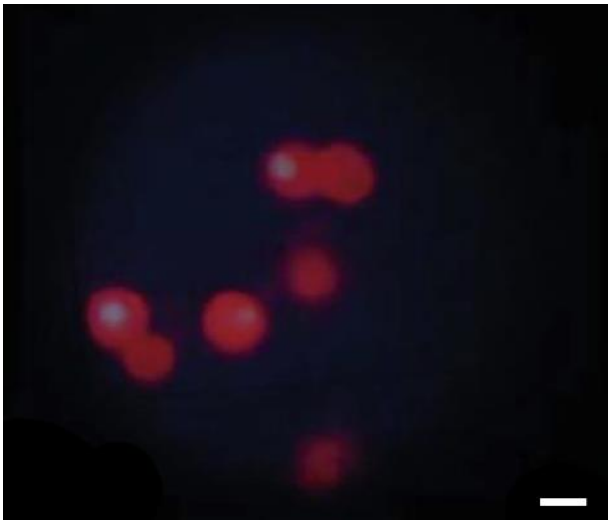


Figure 1: Autofluorescence images showing healthy (red) zooxanthellae. Scale bars = 5 μm.

3.4 Bleaching quantification

In accordance with Siebeck et al. (2006), a colorimetric evaluation was carried out analyzing digital photographs of corals taken at the beginning of the experiment (T_0), after 18 hours (T_{18}), and at the ending of the experiment (T_{42}). Photographs were taken under identical illumination using a digital camera (Canon EOS 400D, Canon Inc., Tokyo, Japan), on a white background with a scale meter. They have been subsequently analyzed through a photo-editing software to quantify color composition of Cyan, Magenta, Yellow and Black (CMYK). Color measures were taken covering the whole area of the coral,

avoiding borders and extremities. Levels of bleaching were measured as the difference between the corals color at the beginning of the experiments (T_0) and after 18 (T_{18}) and 42 (T_{42}) hours of exposure to the different treatments. For each fragment 120 random measurements were made (360 measurements for each treatment), covering the whole surface. Scores of the degree of bleaching were attributed to the average values obtained by mean of a mathematical function (Tab. 2) and according to a scale organized in ranks (0% to >30%), i.e., from "no visible coral bleaching" (0-10%) to "severe bleaching" (>30%). The percentage of bleaching is given out by the following equations:

$$B_{t0-t18} = \left(1 - \frac{S_{t18}}{S_{t0}}\right) * 100$$

$$B_{t0-t42} = \left(1 - \frac{S_{t42}}{S_0}\right) * 100$$

Where:

- B is the degree of bleaching, accompanied by the considered time interval
- S is the sum of the average values C, M, Y and K for each sample, and the respective sampling time is indicated in subscript.

Table 2: Degree of bleaching and relative severity scale for *Seriatopora caliendrum* fragments.

Degree of bleaching (%)	Severity of bleaching
0-10	No visible bleaching, no color variation
11-15	Slight bleaching, mild color variation
16-20	Moderate bleaching, remarkable color variation
21-30	Strong bleaching, presence of completely bleached areas
>30	Severe bleaching, surface of the coral is mainly bleached

3.5 Prokaryotic and viral abundance in seawater

Prokaryotic and viral abundance in seawater samples was determined according to the protocol described by Noble and Fuhrman (1998). Seawater samples (5 mL) were collected from treated and control systems immediately

after the addition of sunscreens and ingredients (T_0), and after18 (T_{18}) and 42 (T_{42}) hours from the beginning of the experiment. After the collection, seawater samples were stored at -20 °C until the subsequent analysis. This procedure consisted in filtration onto membranes with 0.02 μm pore size (Anodisc Whatmann; ø 25 mm; Al_2O_3), which were then stained using 100 μL of SYBR Gold (stock solution diluted 1:5000). The membranes were incubated in the dark for 20 minutes, washed three times with 3 mL of prefiltered Milli-Q water each time, and set on glass slides with 20 μL of 50% phosphate buffer (6.7 mM phosphate, pH 7.8) and 50 % glycerol (containing 0.5% ascorbic acid), both below and on top of the filter. Slides were stored at -20 °C. Prokaryotes and viruses counts were obtained by epifluorescence microscopy (Zeiss Axioskop 2; Carl Zeiss Inc., Jena, Germany). For each slide, at least 20 microscope fields were observed, and bacteria size was evaluated using the meter scale on the lens, to classify them as small (<0.5 μm), medium (between 0.5 and 1 μm) or large (>1 μm).

Different impact levels were established to viral and prokaryotic enrichment in the seawater in which the corals were immersed. The viral and prokaryotic enrichment was calculated by the ratio between the abundances at the sampling times (T_{18} and T_{42}) and at the beginning (T_0) of the experiment of each component and compared with the control values. Viral and prokaryotic

enrichment ≤3 was defined as slight (i.e., associated with microbial proliferation due to the presence of a source of organic matter represented by the solar products/ingredients introduced into the systems), between 3.1 and 5 moderate and >5.1 severe or very severe (potentially attributable to coral/symbiont microalgae degradation; Corinaldesi et al., 2017).

3.6 Statistical analysis

Differences in the investigated variables between controls and treatments were assessed using permutational analyses of variance (PERMANOVA; Anderson, 2005; McArdle and Anderson, 2001) on square root transformed data. The design included two fixed factors (time and treatment). When significant differences respect to the control systems were encountered ($p<0.05$) post-hoc pairwise tests were also carried out. Statistical analyses were performed using PRIMER 6 (Clarke and Gorley, 2006).

3.7 Overall impact

In order to formulate an overall judgment on the effect of sunscreen products and ingredients on coral fragments, a different weight in percentage

of the individual variables was attributed on the basis of their importance with respect to the potential impact on organisms: 30% for zooxanthellae release (2.1), 45% for the degree of bleaching (3.15), 18% for virus enrichment in seawater (1.26) and 7% for prokaryote enrichment (0.49). Virus enrichment was considered more important than prokaryotic enrichment because previous studies have shown that viral infection induces bleaching of corals exposed to sunscreens and filters (Danovaro et al., 2008).

A value on a scale ranging from 0 (no significant impact) to 4 (severe impact; Tab. 3) was attributed to each variable (zooxanthellae release, degree of bleaching, virus enrichment, and prokaryotic enrichment) for all tested products. The overall impact of sunscreens was calculated as the average of the values assigned to the single variables, weighted on their coefficient.

Table 3: Severity of impact on different variables (zooxanthellae release, degree of bleaching, virus enrichment, and prokaryotic enrichment) for *S. caliendrum*, and corresponding value assigned.

Severity of impact	Value
No impact	0
Slight	1
Moderate	2
Strong	3
Severe	4

4. RESULTS

4.1 Abundance of zooxanthellae released in seawater surrounding corals

Figures 2 and 3 show the number of total zooxanthellae released into the seawater surrounding coral fragments in the control (untreated systems) and in systems treated with sunscreen products and ingredients (50 µL L^{-1}) during the 42 hours of the experiment.

The abundance of zooxanthellae released into the seawater surrounding coral fragments exposed to the sunscreen products S3, S4, S5, S6, S8, S15, S17, S18 was not significant, or significantly lower than the control, at the beginning of the experiment (T_0) and after 18 (T_{18}) and 42 (T_{42}) hours of exposure (Fig. 2).

The sunscreen product S7 did not cause a significant increase compared to the control of the zooxanthellae abundance at the beginning of the experiment (T_0), but the values significantly increased after 18 hours (T_{18}) of exposure (Fig. 2; $p<0.05$). On the contrary, at the end of the experiment S7 did not show a significant increase in the number of zooxanthellae released compared to the control; despite this, the total abundance of zooxanthellae released into the seawater surrounding coral fragments reached values about 2 times higher than the control (Fig. 2). The variability encountered in S7 was

due to the complete bleaching of only one of the replicates (as shown in Fig. 8).

Similarly, S16 showed no significant variations of the zooxanthellae abundance in comparison to the control at the beginning (T_0) and at the end of the experiment (T_{42}), while showing a significant increase ($p<0.05$) in the abundance of the symbiotic microalgae released after 18 hours of exposure (T_{18}), reaching values almost six times higher than the control systems (Fig. 2).

Among the cosmetic ingredients, Sensamone P5 (S19) showed no significant difference in the abundance of the zooxanthellae released into the seawater surrounding coral fragments compared to the control systems at the beginning (T_0) and at the end (T_{42}) of the experiment, but after 18 hours (T_{18}) we observed a significant increase of the zooxanthellae released, compared to the control (Fig. 3; $p <0.05$).

A high release of zooxanthellae ($p<0,05$) into seawater surrounding coral fragments was observed after exposure to Hydropuntil (S20) in respect to the control, at the beginning (T_0) of the experiment and after 18 (T_{18}) and 42 (T_{42}) hours of exposure (Fig. 3). In particular, immediately after the addition of the treatment (S20), an abundance of the zooxanthellae released about 2.72 times higher than in the control was observed (Fig. 3).

Similarly, coral fragments treated with Senseryn (S21) showed a significant increase of the zooxanthellae released in the surrounding seawater significantly higher than the control at the beginning of the experiment (T_0; $p<0.05$) and after 18 hours (T_{18}; $p<0.01$). On the contrary, at the end of the experiment (T_{42}) no significant difference was observed in comparison with the control system.

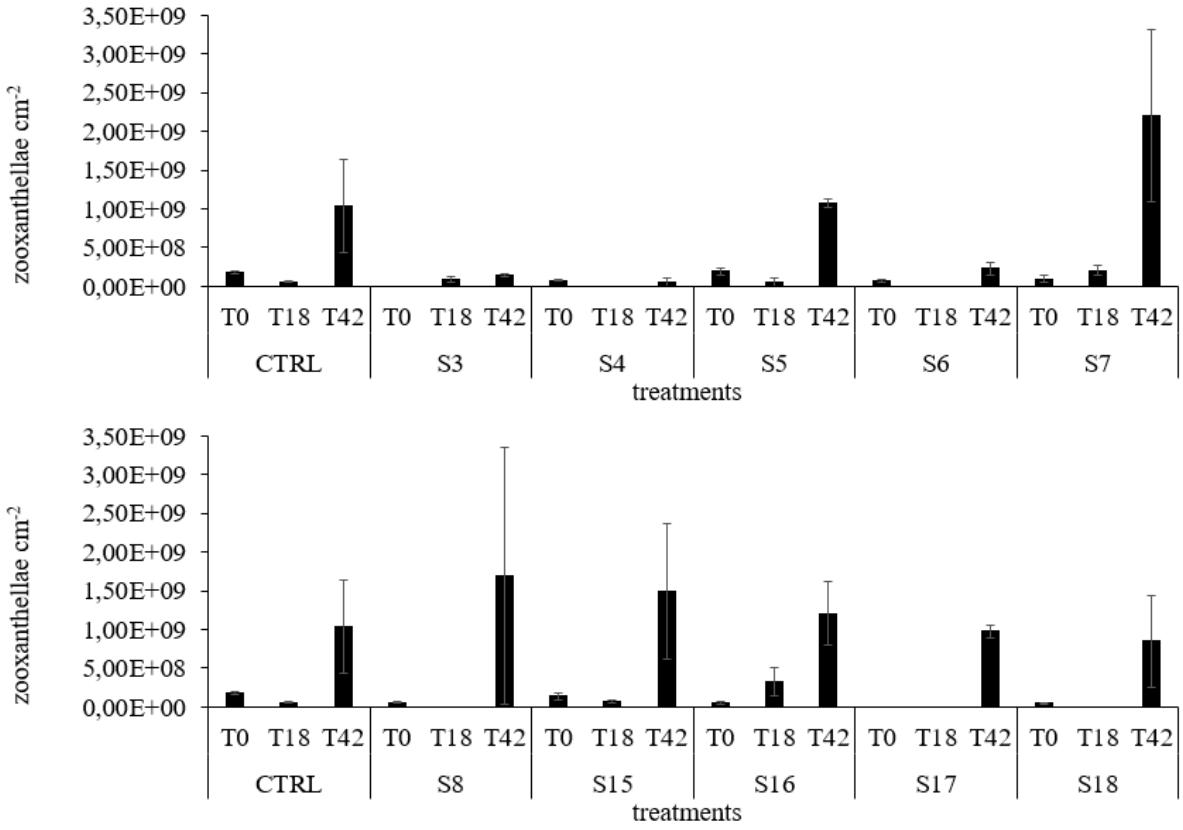


Figure 2: Number of zooxanthellae released per cm^{-2} into the seawater surrounding *Seriatopora caliendrum* fragments exposed to different brands of sunscreen products (S3-S18) during 42 hours of experiment. ± ER

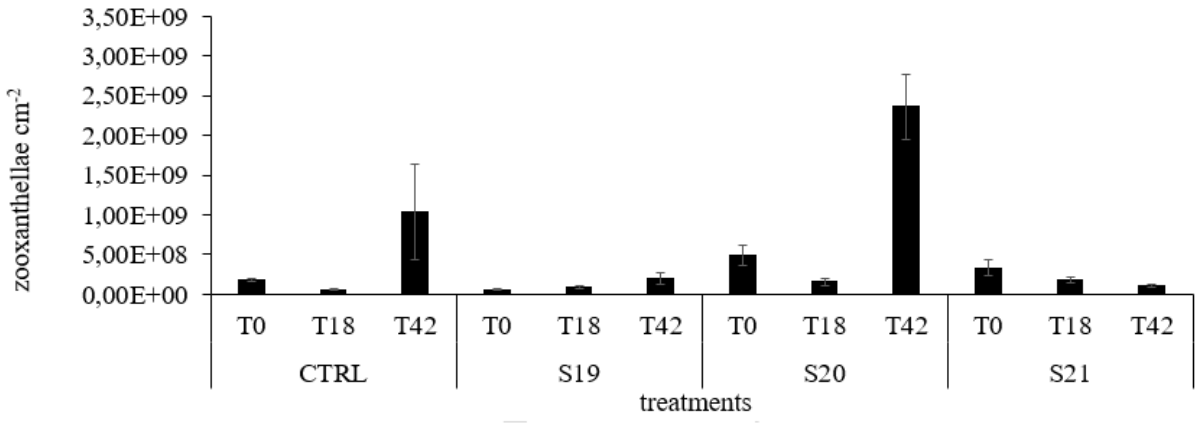


Figure 3: Number of zooxanthellae released per cm^{-2} into the seawater surrounding *Seriatopora caliendrum* fragments exposed to different ingredients of personal care products (S19-S21) during 42 hours of experiment. ± ER

4.2 Bleaching quantification

The results of the colorimetric analysis conducted on the *S. caliendrum* coral fragments treated with sunscreen products, ingredients and controls (untreated systems) during the 42 hours of the experiment are summarized in Table 3. All treatments and control systems did not show a change of the colorimetric variables (CYMK) from the beginning (T_0) to 18 hours (T_{18}) of the experiment, with values of the degree of bleaching comprising between 0% and 4%, falling into category of "no visible bleaching" (Fig. 4 and Fig. 5).

The control systems (untreated coral fragments), at the end of the experiment (T_{42}) resulted in a degree of bleaching of 7.3%, indicating no visible bleaching (Tab. 4; Fig. 6).

Among the sunscreens, S3, S4, S15, S17 and S18 showed no significant change in the colorimetric variables (CYMK) compared to the control at the end of the experiment (T_{42}), with values of the degree of bleaching lower than 10%.

Instead, *S. caliendrum* coral fragments treated with sunscreen products S5 and S19 showed a slight degree of bleaching (Fig. 4), between 10% and 15%, as reported in Table 3.

The coral nubbins exposed to S6, S8 and S16 showed a change in the colorimetric variables (CYMK) from the beginning (T_0) to the end (T_{42}) of the experiment, resulting in moderate bleaching with values ranging from 15% to 20% (Tab. 4).

The addition of sunscreen product S7 caused, at the end of the experiment (T_{42}), a strong loss of color in coral nubbins tissue (bleaching degree equal to 25.93%; Tab. 4). In particular, severe bleaching (100% of the nubbin surface) was observed in one of the three coral fragments exposed to this sunscreen product (Fig. 6; Fig. 8).

Similarly, ingredient S21 (Senseryn) caused a noticeable loss of color (degree of bleaching 21.35%) on the coral nubbins surface from the beginning (T_0) to the end (T_{42}) of the experiment (Fig. 7), especially in one of the three coral fragments that showed about 40% of its surface with evident signs of strong bleaching (Fig. 7; Fig. 8).

The highest degree of bleaching was observed in *S. caliendrum* fragments exposed to ingredient Hydropuntil (S20), which recorded a value of 38.55%, falling into the category of severe bleaching (Tab. 4). In particular, one of the nubbins treated with S20 was totally bleached (100% of the coral nubbin surface), and another one showed signs of bleaching on about 40% of the coral surface (Fig.7; Fig. 8).

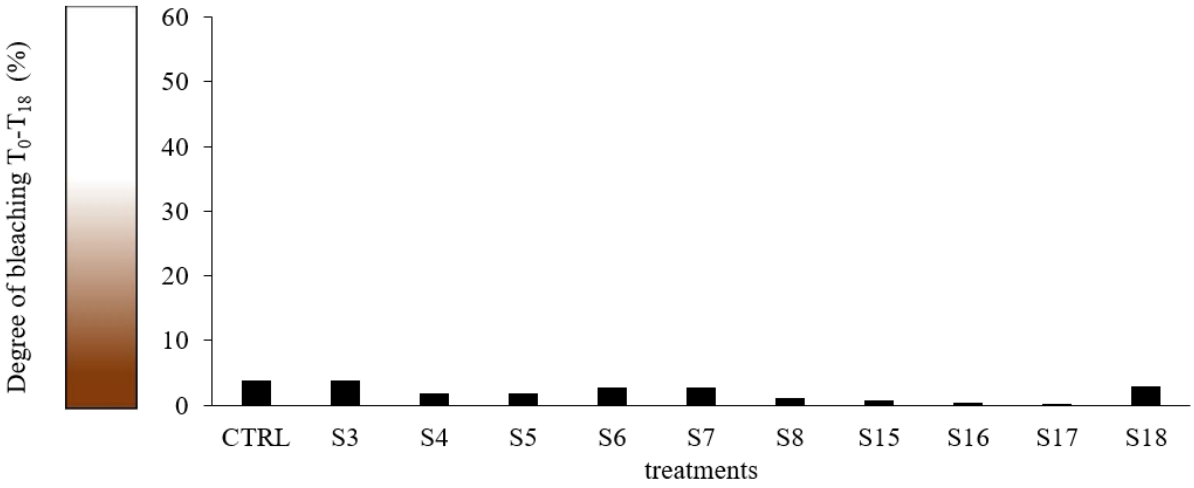


Figure 4: Degree of bleaching of *S. caliendrum* fragments after 18 hours of exposure to sunscreen products (S3-S18) and untreated systems (control).

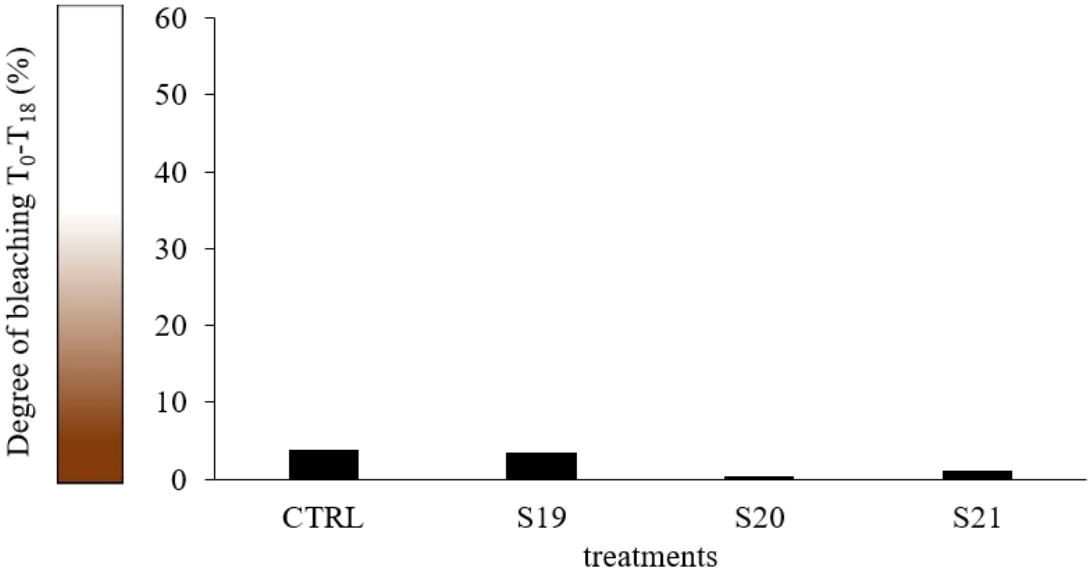


Figure 5: Degree of bleaching of *S. caliendrum* fragments after 18 hours of exposure to ingredients (S19-S21) and untreated systems (control).

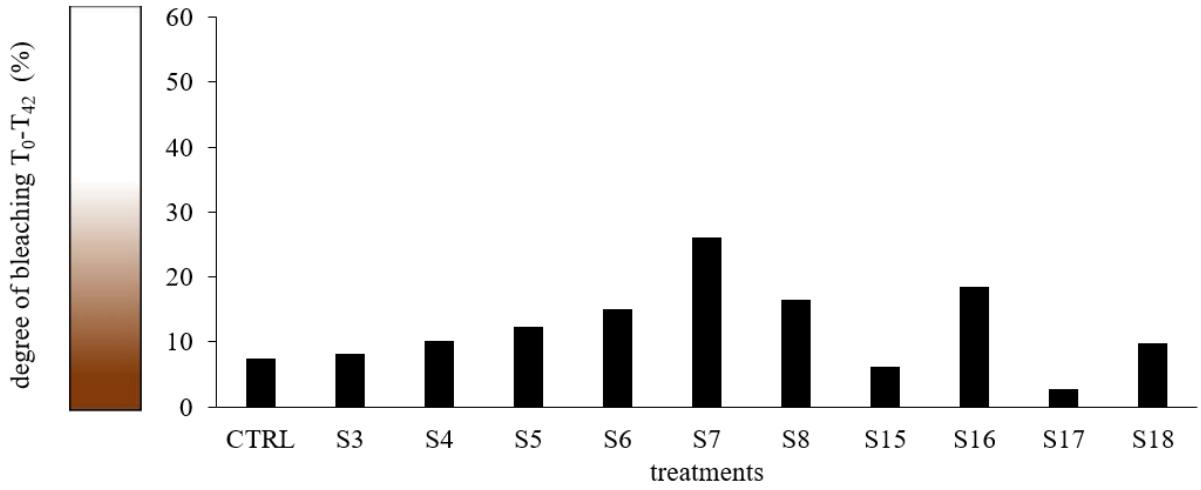


Figure 6: Degree of bleaching of *S. caliendrum* fragments after 42 hours of exposure to sunscreen products (S3-S18) and untreated systems (control).

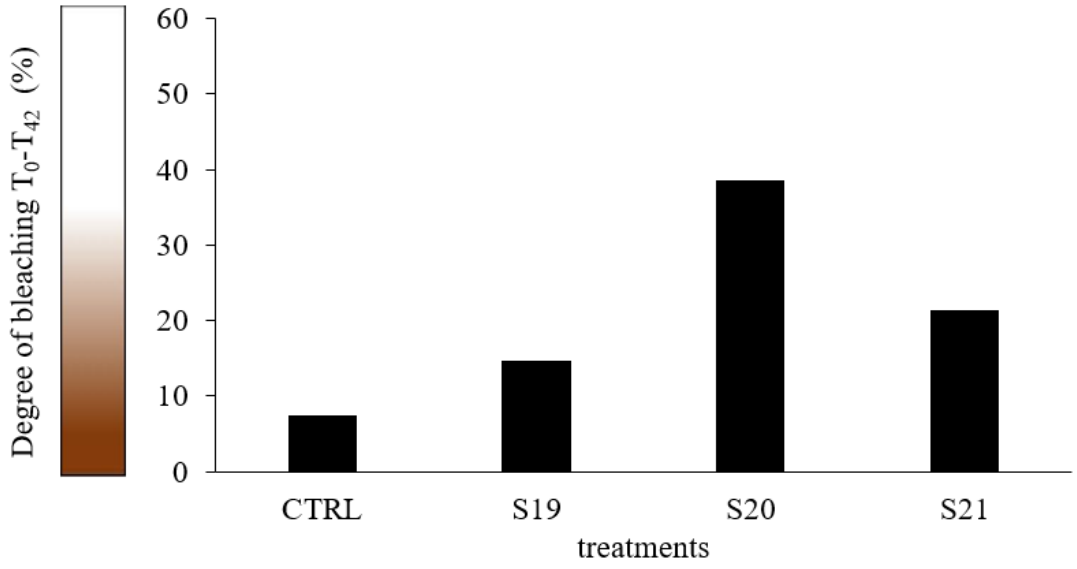


Figure 7: Degree of bleaching of *S. caliendrum* fragments after 18 hours and 42 hours of exposure to ingredients (S19-S21) and untreated systems (control).

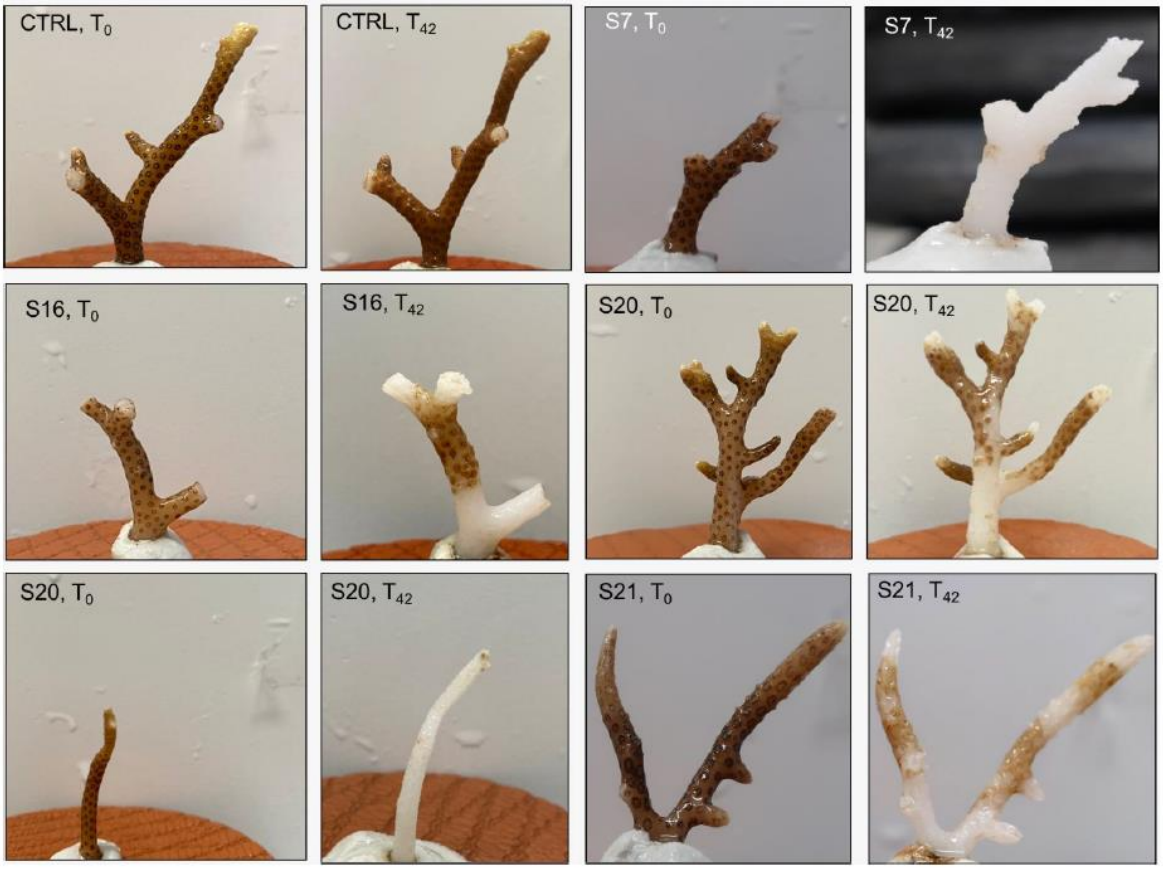


Figure 8: Photographs of control corals (CTRL, i.e., without the addition of sunscreen products and ingredients) and *S. caliendrum* coral fragments exposed to S7, S16, S20 and S21 sun products/ingredients at the beginning (T_0) and after 42 hours (T_{42}) of the experiment where the signs of bleaching were visible.

Table 4: Mean values of the degree of bleaching after exposure of *Seriatopora caliendrum* fragments for 42 hours to different sunscreen products (S3-S18) and ingredients (S19-21), and relative severity scale.

Treatment	Degree of bleaching (%)	Severity of bleaching (%)
CTRL	7.31	0-10, no visible bleaching
S3	8.14	0-10, no visible bleaching
S4	9.99	0-10, no visible bleaching
S5	12.31	11-15, slight bleaching
S6	17.28	16-20, moderate bleaching
S7	25.93	21-30, strong bleaching
S8	16.47	16-20, moderate bleaching
S15	6.04	0-10, no visible bleaching
S16	18.46	16-20, moderate bleaching
S17	2.66	0-10, no visible bleaching
S18	9.72	0-10, no visible bleaching
S19	14.55	11-15, slight bleaching
S20	38.55	>30, severe bleaching
S21	21.35	21-30, strong bleaching

4.3 Prokaryotic and viral abundance in seawater

4.3.1 Prokaryotic abundance

Figures 9 and 10 show the prokaryotic abundance in the seawater surrounding coral fragments in the control (untreated systems) and in systems treated with sunscreen products and ingredients (50 $\mu L\ L^{-1}$) during the 42 hours of the experiment.

At the beginning of the experiment (T_0), all treated systems recorded values of prokaryotic abundance comparable to the control (S3, S5), or even significantly lower (S4, S6, S7, S8, S15, S16, S17, S18, S19, S20, S21; Fig. 9; Fig. 10).

Sunscreen products S3 and S15 did not cause significant variations in prokaryotic abundance in the seawater surrounding coral fragments after 18 hours (T_{18}) of the experiment compared to untreated systems (control); conversely, they determined a significant ($p<0.01$) prokaryotic enrichment after 42 hours (T_{42}) of exposure.

The sunscreen products S4, S17 and S18 did not induce significant variations in prokaryotic abundance into the seawater surrounding coral fragments after 18 (T_{18}) and 42 (T_{42}) hours of experiment compared to the control systems (Fig. 9).

On the contrary, S5 determined a significant prokaryotic enrichment ($p<0.01$) into seawater surrounding coral fragments compared to the control after 18 (T_{18}) and 42 (T_{42}) hours of exposure (Fig. 9).

Sunscreen products S6, S8 and S16 showed significantly lower abundances of prokaryotes into seawater surrounding coral nubbins compared to the control after 18 hours (T_{18}) of experiment, but these values significantly increase after 42 hours (T_{42}; $p<0.001$, $p<0.05$ and $p<0.01$ respectively) compared to control systems.

S7 showed significantly lower abundance of prokaryotes in seawater surrounding corals after 18 hours (T_{18}) of the experiment, while at the end of the experiment (T_{42}) no significant difference compared to the control was observed.

Among the ingredients, systems treated with S19 (Sensamone P5) and S20 (Hydropuntil) showed a prokaryotic abundance significantly lower than the control after 18 hours (T_{18}) of exposure (Fig. 10), but no significant difference compared to the control was observed at the end of the experiment.

Ingredient S21 (Senseryn) did not determine significant differences of prokaryotic abundance in seawater surrounding coral fragments compared to

control (untreated systems) after 18 (T18) and 42 (T42) hours of the experiment (Fig. 10).

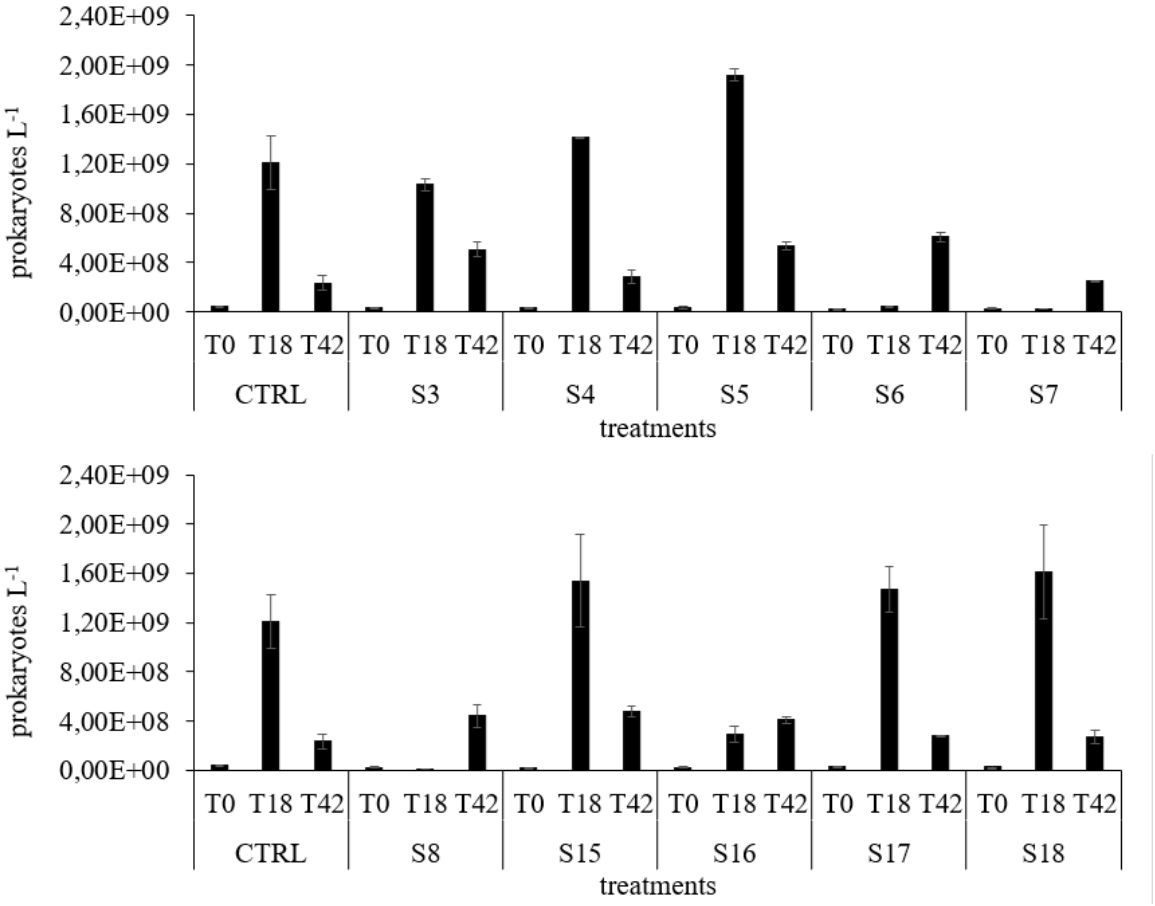


Figure 9: Prokaryotic abundance into seawater surrounding *Seriatopora caliendrum* fragments exposed to different brands of sunscreen products (S3-S18) and untreated systems (control) during 42 hours of experiment. ± ER

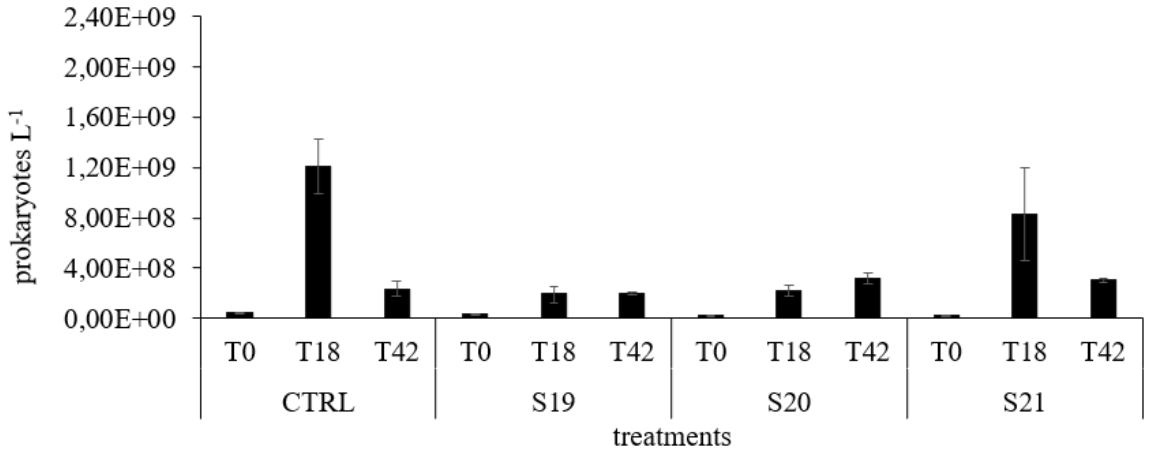


Figure 10: Prokaryotic abundance into seawater surrounding *Seriatopora caliendrum* fragments exposed to different (S19-S21) and untreated systems (control) during 42 hours of experiment. ± ER

4.3.2 Viral abundance

Figures 11 and 12 show the viral abundance into the seawater surrounding coral fragments in the control (untreated systems) and in systems treated with sunscreen products and ingredients (50 µL L^{-1}) during the 42 hours of the experiment.

Systems treated with sunscreens S3, S5, S6, S8, S15 and S17 did not cause significant variations in virus-like particles abundance in comparison to the control during all the experimental time (T_0, T_{18} and T_{42}; Fig. 11).

On the contrary, corals treated with S4 showed a significant increase ($p<0.01$) of the virus-like particles abundance into seawater surrounding coral fragments in comparison to control systems at the beginning of the experiment (T_0), but no significant differences were observed after 18 (T_{18}) and 42 (T_{42}) hours of the experiment.

Sunscreen product S7 did not cause significant variations of virus-like particles abundance compared to untreated systems (control) at the beginning of the experiment (T_0) and after 42 hours (T_{42}); conversely, a significant increase ($p<0.05$) in the virus-like particles abundance compared to the control was observed after 18 hours (T_{18}) of exposure (Fig. 11).

The addition of sunscreen product S16 did not cause a significant increase in the virus-like particles abundance at the beginning (T_0) and after 18 hours (T_{18}) of the experiments, but these values significantly increase ($p<0.05$) at the end of the experiments (T_{42}) than the control systems (Fig. 11).

Conversely, seawater surrounding corals treated with sunscreen S18 showed significant increase ($p<0.01$) of virus-like particles abundance compared to the control (untreated systems) at the beginning of the experiment (T_0), but no significant differences were observed after 18 (T_{18}) and 42 (T_{42}) hours of exposure than the control (Fig. 11).

Among the ingredients, S19 (Sensamone P5) showed significantly higher ($p<0.05$) values of viral abundance in seawater surrounding coral fragments at the beginning of the experiment (T_0), in comparison with the control (Fig. 12), but no significant differences were observed after 18 (T_{18}) and 42 (T_{42}) hours.

After addition of S20 (Hydropuntil) and S21 (Senseryn) no significant differences on viral abundance than the control systems were observed at the beginning of the experiment (T_0), and after 18 (T_{18}) and 42 (T_{42}) hours of exposure (Fig. 12).

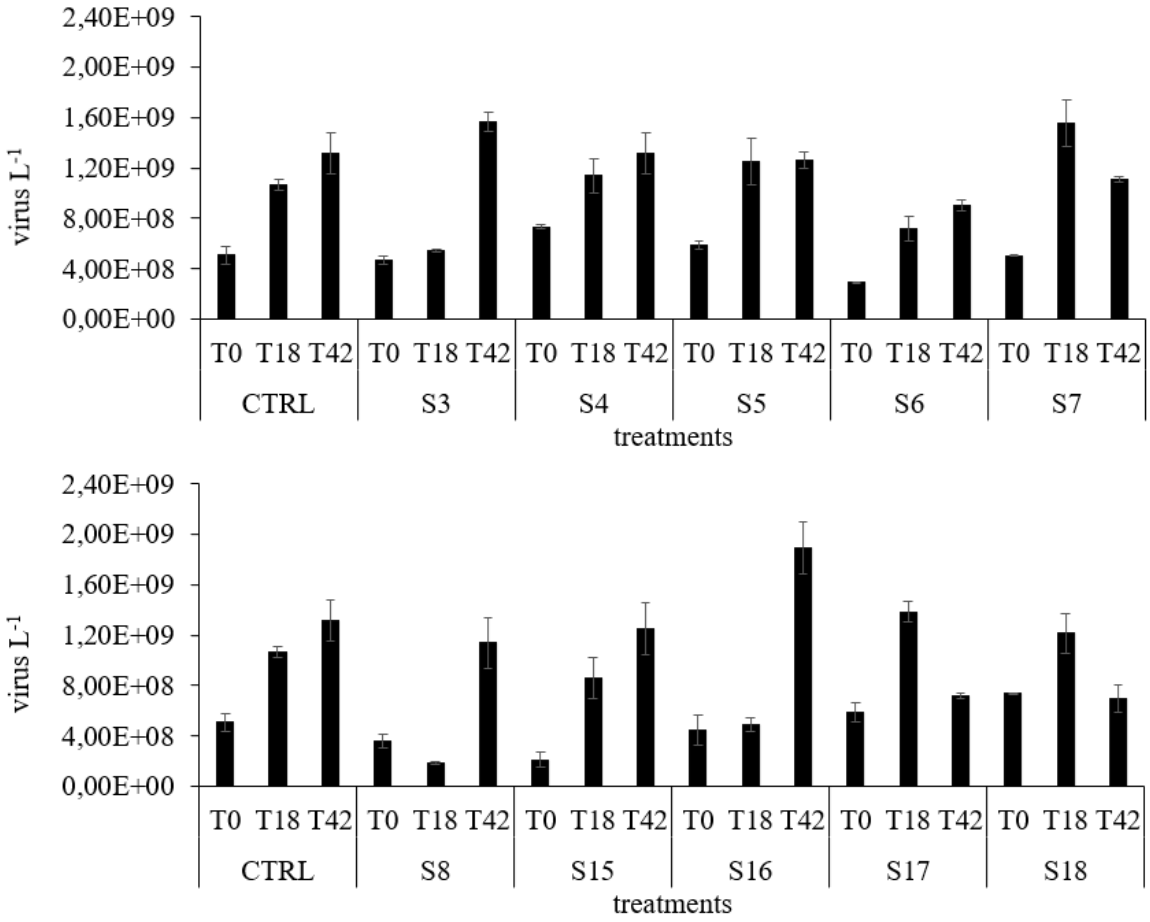


Figure 11: Virus-like particles abundance into seawater surrounding *Seriatopora caliendrum* fragments exposed to different brands of sunscreen products (S3-S18) and untreated systems (control) during 42 hours of experiment. ± ER

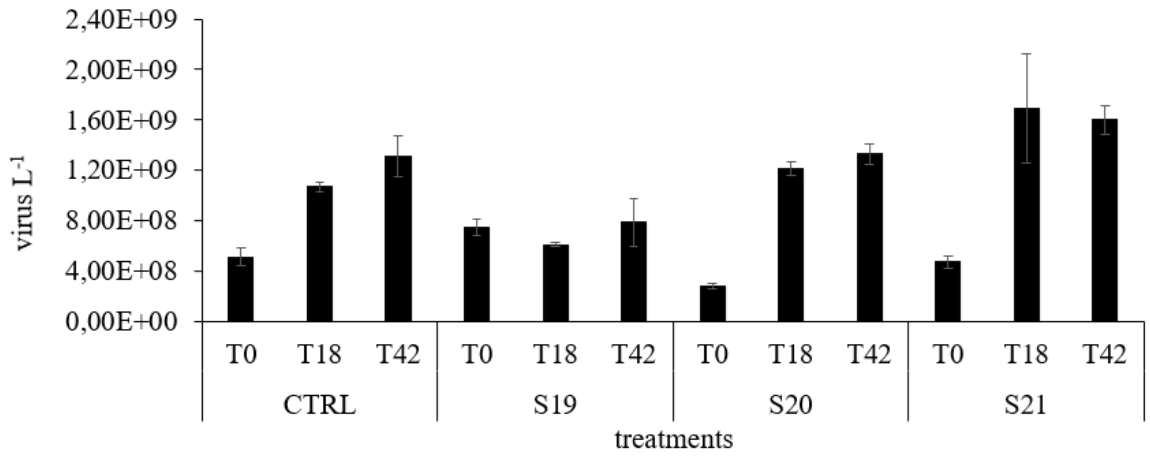


Figure 12: Virus-like particles abundance into seawater surrounding *Seriatopora caliendrum* fragments exposed to different ingredients of personal care products (S19-S21) and untreated systems (control) during 42 hours of experiment. ± ER

4.4 Overall impact

Table 5 shows the results of the impact levels of sunscreen products and ingredients for each variable analyzed and the overall impact level resulting from the integration of all the results as reported in Chapter 3: Materials and Methods.

Sunscreens S3, S4, S15, S17 and S18 did not cause a significant overall impact on *S. caliendrum* fragments within tested variables.

Sunscreens S5, S6 and S8 had slight overall impacts on coral fragments.

Coral fragments treated with sunscreens S7 and S16 underwent a moderate overall impact.

Natural active ingredients induced the worse negative effects among the tested products: S19 induced slight impact, while S20 and S21 caused strong and moderate overall impacts, respectively.

Table 5: Results of the impact of each sunscreen products (S3-S18) and active ingredients (S19-S21) tested for each variable analyzed (release of symbiotic microalgae, bleaching, viral and prokaryotic enrichment) and overall impact. Legend is reported below the table.

Treatments	Zooxanthellae release (2.1)	Degree of bleaching (3.15)	Viral abundance (1.26)	Prokaryotic abundance (0.49)	**Overall impact**
CTRL	0	0	0	0	**0**
S3	0	0	0	1	**0**
S4	0	0	0	0	**0**
S5	0	1	0	1	**1**
S6	0	2	0	2	**1**
S7	3	3	1	0	**2**
S8	0	2	0	2	**1**
S15	0	0	0	2	**0**
S16	4	2	1	1	**2**
S17	0	0	0	0	**0**
S18	0	0	0	0	**0**
S19	2	1	0	0	**1**
S20	3	4	0	0	**3**
S21	3	3	0	0	**2**

Legend:

No impact	0
Slight impact	1
Moderate impact	2
Strong impact	3
Severe impact	4

5. DISCUSSION

The release of coral symbiotic zooxanthellae, known as coral bleaching, has negative impacts on biodiversity and functioning of reef ecosystems and their production of goods and services (Burke et al., 2011; Wild, 2004). Also, the degree of bleaching of coral tissues is directly correlated with zooxanthellae release (Brown, 1997).

Previous studies (Wijgerde et al, 2020; He et al., 2019; Corinaldesi et al., 2018; Danovaro et al., 2008) have demonstrated that sunscreen products and their ingredients have a rapid effect on hard corals and cause bleaching by damaging the symbiotic zooxanthellae.

Our results are in line with those previously obtained. Among 13 treatments tested on hard coral between sunscreen products and active ingredients, 8 formulations caused negative effects on *S. caliendrum* fragments, with an impact ranging from slight to strong. However, the different brands of sunscreens and active ingredients tested caused different responses in term of the release of zooxanthellae and degree of bleaching.

In particular, the addition of sunscreens S7 and S16 resulted in the high release of symbiotic algae after 18 hours (T_{18}) of the beginning of the experiment, with values of release 4 and 6 times greater than the control. These

findings were confirmed by the colorimetric analysis of the coral surface where the exposure to S7 and S16 caused a strong and moderate bleaching, respectively. In addition, we observed that S5, caused a slight loss of color on coral fragments surface, while sunscreens S6 and S8 caused a moderate bleaching on *S. caliendrum*.

On the other hand, no significant release of zooxanthellae and no visible bleaching were observed in *S. caliendrum* coral fragments exposed to sunscreen products S3, S4, S15, S17 and S18 within 42 hours of experiment.

The different impacts observed in systems exposed to sunscreen products may be due to the different formulation in terms of the concentrations of single ingredients contained by the tested brands. In our experiment, sunscreen products all have a similar formulation in terms of the organic UV filters used and ingredients, but the percentages of these ingredients within the composition are different.

The most severe effects were observed after the addition of the "natural" active ingredients. Hydropuntil (S20) and Senseryn (S21) caused a high release of zooxanthellae during the 42 hours of exposure, also reflected on a severe and strong bleaching, respectively. In particular, S20 caused a total bleaching on corals surface within 42 hr of the experiment, with a strong impact on *S. caliendrum*. Corinaldesi et al. (2018) obtained similar results from zinc oxide

(ZnO) exposure on *Acropora* spp. This negative effect may be due the formulation of this ingredient (S20) that contains potassium sorbate, a chemical that in previous studies has been proved to negatively affect marine organisms (Peng et al., 2019; Chen et al., 2017), and has also an antifouling effect (Blustein et al., 2009). Senseryn (S21), instead, contains hops (*Humulus lupulus*, Linnaeus 1753) extracts, which may have sedating properties (Schiller et al., 2006).

Also, active ingredient Sensamone P5, which contains a biomimetic peptide based on a component of sea anemone venom, caused a slight impact on *S. caliendrum*. In particular, a significant release of symbiotic microalgae was observed after 18 hours of exposure and caused a slight bleaching of the coral fragments surface.

Unfortunately, there are no other studies that evaluate the effect of these active ingredients, defined "natural smart ingredients", on marine organisms. This is the first study that evaluates the potential impact of these three active ingredients, widely used in cosmetics and sun products, on the coral reef ecosystem.

Organic products should also be biodegradable and, hence, have a low environmental impact. However, not all natural substances are degradable in the short term or have a low environmental impact (Dayan and Kromidas,

2011). For example, caffeine is a natural substance, but several studies have shown that it has a negative effect on marine organisms (Pires et al., 2016; Jiangn et al., 2014). Therefore, cosmetic ingredients defined "eco-compatible" or "natural" should be tested on marine organisms, or at least on key-species, such as corals.

Our results are consistent with those reported by Pawlowski et al. (2021), who highlighted that the organic UV filters also contained in sunscreens products tested in our study (S3-S18) resulted as the least impactful among the 24 substances investigated by Pawlowski et al. (2021). Indeed, we observed no severe alterations, contrarily to previous studies about sunscreens impact on the marine environment (He et al., 2019; Danovaro et al., 2008). Nevertheless, 5 out of the 10 sunscreen formulations caused a slight to moderate impact, with negative implications on reef ecosystems. Specifically, sunscreens S5, S6, S7, S8 and S16 caused a slight to moderate impact on *S. caliendrum*. This effect may be due to a higher concentration within these formulations of organic UV filters, adjuvants and preservatives than the sun products S3, S4, S15, S17 and S18, which, on the contrary, did not cause an impact on hard coral and its symbiotic microalgae.

Our results are consistent with the ones obtained by Corinaldesi et al. (2018), who observed a prokaryotic and viral enrichment in seawater

surrounding corals after exposure to sunscreens, in particular in the systems treated with S16. This can be explained by the production of coral mucus, which is rich of selected bacterial communities (Shnit-Orland and Kushmaro, 2009), in response to chemical stressors (Vacelet and Thomassin, 1991), or by the leverage for prokaryotes resulting from the direct nourishment of organic matter contained in sunscreens (Kujawinski, 2011).

6. CONCLUSIONS

Overall, our findings indicate that most of the sunscreen products tested can rapidly affect hard corals causing bleaching and release of symbiotic zooxanthellae.

These effects depend both on the different ingredients and the percentage of these ingredients contained in the final formulation. The most severe effects were determined by ingredients defined as “natural” or reported to be eco-friendly, suggesting that such products are not actually tested on marine life, suggesting that choosing the best ingredients on the basis of previous results, as several cosmetic companies do, is not enough to define the formulation as eco-friendly.

On the basis of our results, we can conclude that a cosmetic product defined “eco-friendly” or “biodegradable” before entering the market should be evaluated with specific biodegradability tests and rigorous studies on marine organisms belonging to different levels of the food web.

APPENDIX

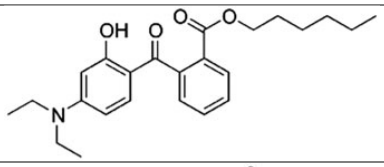
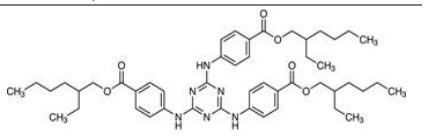
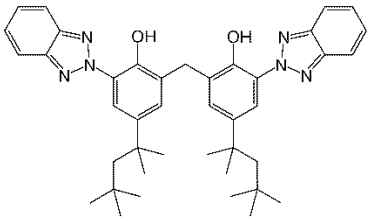
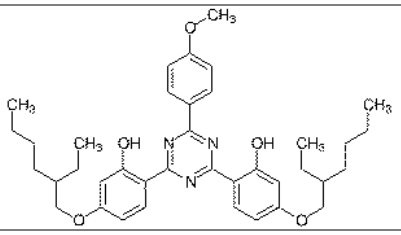
DHHB	Diethylamino hydroxybenzoyl hexyl benzoate	$C_{24}H_{31}NO_4$	
EHT	Ethylhexyl triazone	$C_{48}H_{66}N_6O_6$	
MBBT	Methylene bis-benzotriazolyl tetramethylbutylphenol	$C_{41}H_{50}N_6O_2$	
BEMT	Bis-ethylexyloxyphenol methoxyphenyl triazine	$C_{38}H_{49}N_3O_5$	

Table 3: From the left, abbreviation, international nomenclature, chemical formula, and molecular structure of the organic UV filters contained in tested sunscreen products. (PubChem)

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