Toxicity of UV filters on marine bacteria: Combined effects with damaging solar radiation

Clément Lozano a,b, Sabine Matallana-Surget b,⁎, Justina Givens a, Salomé Nouet a, Louise Arbuckel b, Zacharie Lambert a, Philippe Lebaron a,⁎

a Sorbonne Université, CNRS, Laboratoire de Biodiversité et Biotechnologies Microbiennes, USR3579, Observatoire Océanologique, 66650 Banyuls-sur-mer, France
b Division of Biological and Environmental Sciences, Faculty of Natural Sciences, Stirling University, United Kingdom

HIGHLIGHTS
• Five organic UV filters were tested for the first time on 27 marine bacteria.
• Seven bacteria demonstrated sensitivity against at least one UV filter.
• Octinoxate is the most toxic UV filter, affecting 5 out of 7 sensitive species.
• The physiological state plays a key role in the bacterial sensitivity to UV-filters.
• Solar radiation modulates UV filters toxicity.

ABSTRACT
Organic UV filters are of emerging concern due to their occurrence and persistence in coastal ecosystems. Because marine bacteria are crucial in the major biogeochemical cycles, there is an urgent need to understand to what extent these microorganisms are affected by those chemicals. This study deciphers the impact of five common sunscreen UV filters on twenty-seven marine bacteria, combining both photobiology and toxicity analysis on environmentally relevant species. Seven bacteria were sensitive to different organic UV filters at 1000 μg L−1, including octinoxate and oxybenzone. This is the first report demonstrating inhibition of bacterial growth from 100 μg L−1. None of the UV filters showed any toxicity at 1000 μg L−1 on stationary phase cells, demonstrating that physiological state was found to be a key parameter in the bacterial response to UV-filters. Indeed, non-growing bacteria were resistant to UV-filters whereas growing cells exhibited UV filter dependent sensitivity. Octinoxate was the most toxic chemical at 1000 μg L−1 on growing cells. Interestingly, photobiology experiments revealed that the toxicity of octinoxate and homosalate decreased after light exposure while the other compounds were not affected. In terms of environmental risk characterization, our results revealed that the increasing use of sun blockers could have detrimental impacts on bacterioplanktonic communities in coastal areas. Our findings contribute to a better understanding of the impact of the most common UV filters on bacterial species and corroborate the importance to consider environmental parameters such as solar radiation in ecotoxicology studies.

⁎ Corresponding authors.
E-mail addresses: sabine.matallanasurget@stir.ac.uk (S. Matallana-Surget), philippe.lebaron@obs-banyuls.fr (P. Lebaron).

https://doi.org/10.1016/j.scitotenv.2020.137803
0048-9697/Crown Copyright © 2020 Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
1. Introduction

Organic UV filters are chemicals that absorb UVB (280–315 nm) and/or UVA (315–400 nm) (Shaath, 2010). Due to their absorbing properties, UV filters are the main components of sunscreen, and are also found in industrial products such as paints and plastics as stabilizing agents. UV filters are of emerging concern due to their large production volumes, the increasing coastal tourism, their persistence related to their chemical properties, and their toxicity (Raineri et al., 2017). Previous studies reported the occurrence of UV filters ranging from nano to microgram per liter in different biotopes (rivers (Kameda et al., 2011; Fent et al., 2010), lakes (Balmer et al., 2005; Fagervold et al., 2019; Langford et al., 2015), coastal waters (Fagervold et al., 2019; Tovar-Sánchez et al., 2013; Apel et al., 2018; Sánchez Rodríguez et al., 2015; Tsui et al., 2019; Sankoda et al., 2015) and sediments (Kameda et al., 2011; Fagervold et al., 2019; Mitchelmore et al., 2019)) but also demonstrated their bioaccumulation and toxic effects on several aquatic organisms (algae (Mao et al., 2018; Seoane et al., 2017), coral (Mitchelmore et al., 2019; He et al., 2019; Tsui et al., 2017), benthic mollusks (Giraldo, 2017), fishes (Araújo et al., 2018), dolphin (Alonso et al., 2015; Gago-Ferrero et al., 2019), marine bacteria (algal (Mao et al., 2017) Zhang et al. (2017) showed that benzophenone derivatives inhibit the growth of Vibrio fischeri. The authors observed a linear relationship between the LogKow and the toxicity of the UV filter. Similarly, the toxicity of benzophenones on Photobacterium phosphoreum was shown to be correlated with their polarity (Liu et al., 2015). Therefore, there is an urgent need to understand to what extent marine bacteria are affected by those chemicals.

Our study investigates for the first time the toxicity of commonly used UV filters, namely BP3, OC, 4-MBC, EHMC and homosalate (HS) on marine heterotrophic bacteria, sampled from the Mediterranean Sea (Banyuls sur Mer, France). For this purpose, twenty-seven environmentally relevant bacteria, were tested for their response against the above-mentioned compounds. Furthermore, dose response analysis

| Name          | CAS n°   | Structure   | Molecular weight | Log Kow |
|---------------|---------|-------------|------------------|---------|
| Benzophenone-3 (BP3) | 131-57-7 | ![Structure](https://example.com/structure) | 228.243 | 3.52 |
| Ethylhexyl methoxy cinnamate (EHMC) | 5466-77-3 | ![Structure](https://example.com/structure) | 290.397 | 5.80 |
| Octocrylene (OC) | 6197-30-4 | ![Structure](https://example.com/structure) | 361.477 | 6.88 |
| 4-Methyl benzylidene camphor (4-MBC) | 36861-47-9 | ![Structure](https://example.com/structure) | 254.367 | 5.92 |
| Homosalate (HS) | 118-56-9 | ![Structure](https://example.com/structure) | 262.344 | 6.16 |

List of bacterial strains used in this study.

| Phyla                  | Bacterial species          | BBCC number |
|------------------------|----------------------------|-------------|
| Actinobacteria         | Arthrobacter aurescens     | 172         |
| Actinobacteria         | Brachybacterium sacelli    | 164         |
| Actinobacteria         | Dietzia maris              | 167         |
| Bacteroidetes          | Algoriphaexus maninitolivora | 266         |
| Bacteroidetes          | Algoriphaexus armithinivora | 48          |
| Bacteroidetes          | Marinbacter dodekensis     | 57          |
| Bacteroidetes          | Oloya marilimos           | 14          |
| Bacteroidetes          | Sabalitrobacter multivorans | 185        |
| Firmicutes             | Bacillus megaterium       | 240         |
| Firmicutes             | Halobacillus dabanensis    | 119         |
| Firmicutes             | Paenibacillus glucanolyticus | 237       |
| α-Proteobacteria       | Eshiblium mobile           | 367         |
| α-Proteobacteria       | Erythrobacter citreus      | 2           |
| α-Proteobacteria       | Erythrobacter nanhaisediminis | 234      |
| α-Proteobacteria       | Paracoccus hibiscisoli     | 192         |
| α-Proteobacteria       | Pelagibacterium halotolerans | 52        |
| α-Proteobacteria       | Phaeobacter inhibens       | 654         |
| α-Proteobacteria       | Pseudoalteromonas agarivorans | 182     |
| γ-Proteobacteria       | Alteromonos genovensis     | 151         |
| γ-Proteobacteria       | Alteromonos marina         | 54          |
| γ-Proteobacteria       | Enterovibrio calvinsi      | 113         |
| γ-Proteobacteria       | Paraglaciocella mesophila   | 6           |
| γ-Proteobacteria       | Pseudoalteromonas hodoensis | 177      |
| γ-Proteobacteria       | Pseudomonas kummingensis   | 268         |
| γ-Proteobacteria       | Rheinheimera baltica       | 75          |
| γ-Proteobacteria       | Vibrio aestuarinus         | 280         |
| γ-Proteobacteria       | Vibrio azures             | 222         |
was carried out for sensitive strains, in order to better understand the range of UV filters toxicity. Interestingly, UV filters toxicity can also be modulated by other key intrinsic or extrinsic parameters that must be taken into account. For instance, both the physiological state (Jaishankar and Srivastava, 2017; Eng et al., 1991) and sunlight can alter the bacterial susceptibility to bactericidal compounds and anthropogenic contaminants (Sakkas et al., 2009; Petersen et al., 2008; Pelletier et al., 1997). Therefore, we also aimed at assessing the bacterial response to UV filters, harvested at both exponential and stationary phases and combined with solar radiation exposure. Overall, this paper presents a comprehensive investigation of the impact of five common UV filters on diverse marine bacterial species, spanning a range of different cellular parameters and UV exposure.

2. Materials and methods

2.1. Chemicals

Five organic UV filters, namely, benzophenone-3 (CAS-No. 131-57-7), ethylexyl methoxy cinnamate (CAS-No. 5466-77-3), octocrylene (CAS-No. 6197-30-4), 4-methylbenzylidene camphor (CAS-No 36861-47-9) and homosalate (CAS-No 118-56-9), were purchased from Sigma-Aldrich (Steinheim, Germany) (Table 1). Stock solutions of UV filters were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, purity >99%) at a concentration of 750 mg L⁻¹ and stored in the dark at room temperature.

2.2. Bacterial strains

A total of 27 bacterial strains from the Banyuls Bacterial Culture Collection (BBCC, https://collection.obs-banyuls.fr/catalogue.php) was used in this study (Table 2). Species were selected to ensure environmentally relevant diversity. Bacterial strains were kept at −80 °C in marine broth 2216 (DIFCO, United States) with 35% glycerol. Bacteria were grown on marine agar plates. After 24–48 h incubation, colonies were suspended and grown aerobically on a rotary shaker (110 rpm) at 25 °C in artificial seawater with 3 mM o-glucose, vitamins, and trace elements (ASW-G) (Eguchi et al., 1996). Cultures were performed in triplicates.

**Fig. 1.** Schematic drawing of the experimental design used to study the combined effect of UV filters and solar radiation on exponential (A) and stationary phase bacteria (B).
2.3. Toxicity of UV filters

The response of 27 bacterial strains (Table 2) against UV filters (1000 μgL⁻¹) was assessed in minimum growth medium ASW-G. Bacterial growth was monitored in a 24 well plate during 48 h using a microplate reader (Paradigm, Molecular Device, United States) in presence of UV filters, with 1% of DMSO, to enhance the UV filters solubility. UV filters free and DMSO free controls were performed.

2.4. Dose response on bacterial growth

Dose response was further investigated for strains that showed sensitivity to UV filters at 1000 μgL⁻¹. The tested concentrations of UV filters were the following: 0, 100, 200, 500, 1000, 2000 and 4000 μgL⁻¹, in ASW-G with 1% DMSO.

2.5. Combined effect of UV filter and solar radiation

The combined effect of solar radiation and UV filter was assessed on sensitive strains. Exposure to artificial solar light was performed using Oriel (United States) solar simulator, equipped with Hg 1600 W sunlight full spectrum lamp (280–800 nm).

The toxicity of the irradiated and non-irradiated UV filters was assessed on bacteria in stationary phase and exponential phase (Fig. 1). Stationary phase cells were maintained in ASW while growing cells were cultivated in ASW-G (supplemented with 3 mM glucose, trace metals and vitamins). Solar radiation exposure was performed in quartz flasks.

2.5.1. Effect of irradiated UV filters on stationary phase culture

Bacteria were cultured in ASW-G, until stationary phase was reached. Cultures were centrifuged for 10 min at 3000 g and the cell pellets were washed in ASW to remove traces of carbon source. Cells were suspended in ASW with 1000 μgL⁻¹ UV filters, pre-irradiated for 5 h under solar light. Non irradiated molecule and UV filters free medium were used as controls. Flasks were incubated and plated for CFU counting after 0, 5 and 24 h of incubation at 25 °C.

2.5.2. Effect of irradiated UV filters on exponential phase culture

UV filters (1000 μgL⁻¹) in ASW were exposed to artificial solar radiation for 5 h. It is noteworthy to mention that vitamins and glucose were added after exposure to solar radiation in order to prevent UV-induced photoproducts from the growth medium’s components. Media were inoculated with fresh precultures and the optical density (OD) at 620 nm was monitored from 48 to 72 h. Non-irradiated medium and UV filters free medium were used as controls.

2.5.3. Direct effect of solar radiation and UV filters on stationary phase culture

Stationary phase cultures were prepared as described in 2.5.1. Bacteria were submitted to four treatments: (1) ASW + UV filters 1000 μgL⁻¹ in dark condition, (2) ASW + UV filters 1000 μgL⁻¹ exposed to artificial solar light, (3) ASW in dark condition, (4) ASW exposed to artificial solar radiation. Plating for CFU counting was performed after 0, 2.5 and 5 h of exposure to the different treatments.

Table 3

Sensitivity of marine bacteria to different UV filters at 1000 μgL⁻¹. Black colored cells represent toxic UV filters, inducing bacterial growth inhibition.

| Phyla             | Species                     | BBCC number | BP3 | EHMC | OC | 4MBC | HS |
|-------------------|-----------------------------|-------------|-----|------|----|------|----|
| Actinobacteria    | Arthrobacter aurescens      | 172         |     |      |    |      |    |
| Actinobacteria    | Brachybacterium sacelli     | 164         |     |      |    |      |    |
| Actinobacteria    | Dietzia maris              | 167         |     |      |    |      |    |
| Bacteroides       | Algoriphagus mnnitolivorans| 266         |     |      |    |      |    |
| Bacteroides       | Algoriphagus ornithinivorans| 48         |     |      |    |      |    |
| Bacteroides       | Maribacter dokdoensis       | 57          |     |      |    |      |    |
| Bacteroides       | Olesea marilimosa          | 14          |     |      |    |      |    |
| Bacteroides       | Sabulilitobacter multivorans| 185        |     |      |    |      |    |
| Firmicutes        | Bacillus megaterium        | 240         |     |      |    |      |    |
| Firmicutes        | Halobacillus dabanensis     | 119         |     |      |    |      |    |
| Firmicutes        | Paenibacillus glucanolytics| 237         |     |      |    |      |    |
| α-proteobacteria  | Epibacterium mobile         | 367         |     |      |    |      |    |
| α-proteobacteria  | Erythrobacter citreus       | 2           |     |      |    |      |    |
| α-proteobacteria  | Erythrobacter nanaisedimins| 234         |     |      |    |      |    |
| α-proteobacteria  | Paracoccus hibiscisoli      | 192         |     |      |    |      |    |
| α-proteobacteria  | Pelagibacterium halotolerans| 52          |     |      |    |      |    |
| α-proteobacteria  | Phaeobacter inhibens        | 654         |     |      |    |      |    |
| α-proteobacteria  | Pseudoalteromonas agarivorans| 182    |     |      |    |      |    |
| γ-proteobacteria  | Alteromonas genovensis      | 151         |     |      |    |      |    |
| γ-proteobacteria  | Alteromonas marina          | 54          |     |      |    |      |    |
| γ-proteobacteria  | Enterothrobacter calviensis| 113         |     |      |    |      |    |
| γ-proteobacteria  | Paraglacieola mesophila     | 6           |     |      |    |      |    |
| γ-proteobacteria  | Pseudoalteromonas hodoensis| 177         |     |      |    |      |    |
| γ-proteobacteria  | Pseudomonas kumkingensis    | 268         |     |      |    |      |    |
| γ-proteobacteria  | Rheinheimera baltica        | 75          |     |      |    |      |    |
| γ-proteobacteria  | Vibrio aestuarianus         | 280         |     |      |    |      |    |
| γ-proteobacteria  | Vibrio azureus              | 222         |     |      |    |      |    |
Fig. 2. Bacterial growth curves with different concentrations of UV filters. Only species that showed non monotonic response were presented (average ± standard deviation, n = 3).

Fig. 3. Bacterial growth curves with different concentrations of UV filters. Only species that displayed a dose dependent response were presented (average ± standard deviation, n = 3).
2.5.4. Direct effect solar radiation and UV filters on exponential phase culture

Bacterial cultures were grown in Erlenmeyer flasks. Bacteria were harvested at an OD (620 nm) of 0.15 and subsequently transferred into quartz flasks. Bacteria were subjected to four treatments: (1) ASW + UV filters 1000 μg L\(^{-1}\) in dark condition; (2) ASW + UV filters 1000 μg L\(^{-1}\) + solar radiation; (3) ASW in dark condition; (4) ASW + solar radiation. OD (620 nm) was monitored for 5 h.

2.6. Statistical analysis

All experiments were performed in triplicate. The normality of the data was verified using Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) and post-hoc Tukey HSD tests were used to assess the significance of the toxicity between different treatments (p-value < .05).

3. Results and discussion

Our study addressed for the first time, the combined impact of five common UV filters with solar radiation on diverse marine bacteria. In addition to the well-studied UV filters such as octinoxate, oxybenzone, and octocrylene, we provided the original toxicity assessment of homosalate on marine organisms.

3.1. Toxicity of UV filters

We first analyzed the bacterial response to UV filters at a concentration of 1000 μg L\(^{-1}\). For this purpose, we studied a diversity of bacteria from different Phyla, a panel composed of nine γ-Proteobacteria, seven α-Proteobacteria, five Bacteroidetes, three Actinobacteria, and three Firmicutes. A total of 7 out of 27 bacterial species showed a sensitivity to one, or more UV filters at 1000 μg L\(^{-1}\), representing 26% of all tested bacteria (Table 3). OC was toxic to one species. BP3 and HS were deleterious to two species and EHMC was the most toxic affecting five bacterial species. Although 4-MBC toxicity was previously reported (Sieratowicz et al., 2011; Torres et al., 2016; Campos et al., 2017), no sensitivity was observed in this study. It is interesting to note that BP3 only affected gram negative species (Bacteroidetes and Proteobacteria), while EHMC and HS showed toxicity against both gram negative and gram positive bacteria (Actinobacteria and Firmicutes), thus suggesting that UV filters could target different membrane structures according to their physicochemical properties. As previously demonstrated with antibiotics, bactericidal compounds can alter multiple cellular processes, such as DNA replication, by targeting DNA gyrase (Lewin et al., 1991).

![Fig. 4. Indirect effect of solar radiation. Viability of stationary phase bacteria submitted to irradiated or non-irradiated UV filters at 1000 μg L\(^{-1}\), expressed as mean ± standard deviation (n = 3).](image-url)
membrane synthesis, by interfering in the lipid organization of the cell membrane (Müller et al., 2016), or protein synthesis, through binding to ribosomal subunits (Greulich et al., 2015). Therefore, if we hypothesized that UV filters altered the above-mentioned mechanisms, further investigation would be needed to confirm these hypotheses.

Proteobacteria are the most abundant class as they can reach 50% abundance in coastal ecosystem (Coclet et al., 2019). They support multiple functions such as nitrate reduction, denitrification and carbon fixation (Yilmaz et al., 2016). Among all proteobacteria tested, none of the γ-proteobacteria was sensitive to any UV filter and 2 out of 7 α-proteobacteria were sensitive to BP3, including Epibacterium mobile, from the Roseobacter clade. Bacteria from the Roseobacter clade account for 20% of bacterioplanktonic communities in coastal waters (Buchan and Moran, 2005), and display versatile metabolic activities (Azam and Malfatti, 2007). Bacteroidetes are the second most abundant phyla (Coclet et al., 2019) and play an important role in denitrification and organic carbon degradation (Yilmaz et al., 2016). Algoriphagus ornithivorans was the only one out of five Bacteroidetes to be reported as sensitive to HS. Actinobacteria are involved in phosphate uptake and the degradation of cellulose, hemicellulose and chitin (Yilmaz et al., 2016). Within this phylum, Arthrobacter species are known to degrade PAH (Sawulski et al., 2014), polyethylene (Balasubramanian et al., 2010), and metabolize pesticide (Kundu et al., 2019). The growth of two out of three Actinobacteria, including one Arthrobacter species, was altered by UV filters. Lastly, similar trends were observed for Firmicutes that are often found in the gut of marine invertebrates (Li et al., 2018). Taken together, these results suggest that the studied UV filters might affect the growth of key players within coastal microbial communities where UV filters can reach high concentrations (Kim and Choi, 2014; Tsui et al., 2014; Downs et al., 2016).

3.2. Dose response on bacterial growth

Bacteria showing a sensitivity to UV filters at 1000 μg L⁻¹, colored in black in Table 3, were further studied for their dose responses with concentrations ranging from 100 to 4000 μg L⁻¹. The dose response of seven bacteria to different UV filters was tested as follows: Arthrobacter aurescens (BBCC 172) against EHMC and OC; Algoriphagus ornithivorans (BBCC 48) against HS; Dietzia maris (BBCC 167) against EHMC; Epibacterium mobile (BBCC 367) against BP3; Halobacillus dabanensis (BBCC 119) against HS and EHMC; Paenibacillus glucanolyticus (BBCC 237) against EHMC and Pelagibacterium halotolerans (BBCC 52) against BP3 and EHMC (Figs. 2 and 3). We observed different profiles of dose responses. Some strains showed a non-monotonic dose response, which means that the observed inhibition of growth did not increase as we increased the compounds concentrations (Fig. 2). Monotonic and non-monotonic responses were observed for the same given compound, showing the response was species specific.

The growth of P. halotolerans was inhibited by BP3 from 100 to 4000 μg L⁻¹ (Fig. 2). The strain responded to BP3 in a non-monotonic manner with no difference between concentrations from 1000 to 4000 μg L⁻¹.
4000 μgL⁻¹. Similarly, non-monotonic effects were noticed for *A. aurescens* with OC as well as for *D. maris* and *P. glucanolyticus* with EHMC (Fig. 2). Interestingly, a non-monotonic effect was reported when assessing the toxicity of a biocide on *Daphna magna* (De Souza Machado et al., 2017). Indeed, the growth inhibition of *D. magna* was only observed for a given range of lower concentrations, as no effect was reported for the highest concentrations.

In contrast, *A. ornithinivorans*, *H. dabanensis* and *E. mobile* responded in a monotonic dose response manner to HS and BP3, with EC₅₀ of 772, 1000 and 364 μgL⁻¹, respectively (Fig. 3). The dose-response was found to be species and compound-dependent. Indeed, while BP3 exhibited a dose-dependent growth inhibition of *E. mobile* (BBCC 367), a non-monotonic response was observed for *P. halotolerans* (BBCC 52) (Fig. 2), at similar concentrations than previously reported (Mao et al., 2017). BP3 and HS inhibited the growth of *E. mobile* and *A. ornithinivorans*, respectively, whereas HS only delayed the growth of *H. dabanensis*, regardless of the concentration. It should be noted that the concentration of UV filters was not monitored throughout the experiments due to the inherent difficulties of measuring the concentration of UV filters in such a small volume.

Following the guidelines supported by the Regulation (EC) No 1907/2006 regarding the registration, evaluation, authorization and restriction of chemicals (REACH), we achieved environmental risk characterization. Predicted no effect concentration (PNEC), based on EC₅₀ for HS and BP3, and on NOEC for EHMC, divided by arbitrarily defined constants (1000), are respectively 1000, 364 and 200 ng L⁻¹. Taking into account that these values were lower than the ones encountered in the environment (Kim and Choi, 2014; Tsui et al., 2014), a potential ecological risk should be considered.

### 3.3. Combined effect of solar radiation and UV filters

Previous studies demonstrated that UV filters could undergo photo-induced modifications that could alter their toxicity. BP3 displayed phototoxicity on human skin cells (Kim et al., 2018) and the UV-exposed EHMC showed an enhanced toxicity to *Aliivibrio fischeri*, *Daphnia magna* and *Artemia salina* (Gackowska et al., 2018). In this study, two strategies were used to investigate the combined effect of solar radiation and UV filters: (i) indirect effects: bacterial viability was monitored in the dark with UV filters previously exposed to solar radiation during 5 h (Figs. 4 and 5) (ii) direct effects: bacteria were simultaneously exposed to both solar radiation and UV filters (Figs. 6 and 7). In order to understand whether the cellular physiological state plays a role in the bacterial response, experiments were performed on bacteria harvested in stationary (Figs. 4 and 6) and exponential (Figs. 5 and 7) phases.

Regarding the indirect effects of solar radiation on the bacterial response, different patterns were observed depending on the bacterial growth phase. Viability of bacteria harvested in stationary phase was
not affected by pre-irradiated UV filters (Fig. 4). On the contrary, growing bacteria were found to be more impacted by the presence of UV filters (Figs. 4 and 5). In agreement with these results, former studies indicated high photostability of BP3 under artificial light exposure (Rodil et al., 2009; Liu et al., 2011). Pre-irradiation treatments of HS and EHMC were found to reduce their toxicity. Rodil and coworkers (2009) demonstrated that light exposure lowered the toxicity of EHMC on algal culture. To the best of our knowledge, the photostability of HS has never been reported. We provided evidence that its toxicity slightly decreased after exposure to solar radiation, suggesting a photodegradation of the parent compound. Irradiated EHMC was found to produce photoproducts resulting from degradation, isomerization, and dimerization reactions (Rodil et al., 2009; Jentzsch et al., 2016; MacManus-Spencer et al., 2011). In our study, irradiated EHMC did not present any enhanced toxicity in comparison to the non-irradiated molecule. It is noteworthy to emphasize that the viability of *Halobacillus dabanensis* showed a 10-fold increased after 24 h of exposure while the bacterial cells were suspended in glucose free ASW. This growth was consistently observed using a total of 6 replicates in independent experiments and this could be explained by spore forming characteristics of *Halobacillus* species (Liu et al., 2005). Interestingly, it was recently reported that under nutrient limitation, spore forming *Bacillus subtilis* would secrete extracellular factors that kill surrounding cells in order to feed on released nutrients (Gonzalez-Pastor et al., 2003). Hence, *H. dabanensis* might leave its stationary state and re-enter a growth phase by metabolizing nutrients from lysed cells. This consistent latter result needs to be further investigated.

Regarding the direct combined effect of solar radiation and UV filters on stationary phase cells, ANOVA post-hoc Tukey comparison showed no synergetic effect of both stresses on the loss of bacterial viability (Fig. 6). Solar radiation decreased viability of all studied species, except *E. mobile*, known as UVB resistant (Matallana-Surget et al., 2012).

Bacterial growth subjected to combined solar light and UV filters was monitored for 5 h. Fold changes of optical density measured after and before the combined treatment (ODT5/ODT0) were compared for each condition (Fig. 7). The toxicity of BP3 on *E. mobile* slightly increased with light exposure. However, no significant difference was observed between the control without BP3 exposed and the condition with BP3 exposed. EHMC displayed no enhanced phototoxicity on *H. dabanensis*. HS displayed greater toxicity on both *H. dabanensis* and *A. ornithinivorans* (Fig. 7), while pretreatment to solar radiation lowered its effect (Fig. 6). These observations emphasized that the effect of a compound can be enhanced by environmental parameters such as solar radiation.

Overall, our results suggest that bacteria in stationary phase were more resistant to UV filters than in exponential phase. Bacterial resistance in stationary phase was reported for a broad spectrum of antibiotics: fluoroquinolones, beta-lactams (Eng et al., 1991; Spoering and Lewis, 2001), or aminoglycosides (Greulich et al., 2015) that target DNA gyrase, penicillin binding proteins, and ribosomes, respectively. Bacteria entering stationary phase have evolved a well-regulated process allowing to cope with different stresses such as nutrient starvation, temperature change, acidic pH, UV damage or high osmolarity (Hengge, 2011). It was previously reported that the entry in stationary phase induced the expression of key transcription factors, such as stationary...
phase sigma factor in *E. coli* ([Jaishankar and Srivastava, 2017] and *Salmonella* species ([Testerman et al., 2002]), leading to the resistance to multiple stressors.

4. Conclusion

This study is the first to assess the toxicity of UV filters on numerous heterotrophic marine bacteria. Our results demonstrated that 26% of all studied marine bacteria were sensitive to at least one organic UV filter commonly used in sunscreen products. HS toxicity was, for the first time, observed on a marine organism. BrF3 was shown to exclusively affect gram negative while EHMC and HS impacted both gram positive and gram negative species. EHMC, also known as octinoxate, was the most toxic compound in our study, affecting five bacterial species. Phototoxicity analyses revealed a lower toxicity for HS and EHMC after pretreatment to solar radiation while combined exposure highlighted an increase in HS toxicity. None of the UV filters showed any toxicity at 1000 μg L$^{-1}$ on stationary phase cells, demonstrating that physiological state is a key parameter in the bacterial response. In terms of environmental risk characterization, our results revealed that the increasing use of sun blockers could have detrimental impacts on bacterioplanktonic communities in coastal areas.

CRediT authorship contribution statement

Clément Lozano: Conceptualization, Methodology, Investigation, Writing - original draft. Sabine Matallana-Surget: Conceptualization, Methodology, Supervision, Writing - review & editing, Funding acquisition. Justina Givens: Writing - review & editing, Investigation. Salomé Nouet: Investigation. Zacharie Lambert: Investigation. Philippe LeBaron: Conceptualization, Methodology, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

Clément Lozano is the recipient of a 50/50 matching funding scholarship between the University of Stirling (Scotland, United Kingdom) and the University of Sorbonne (France). We thank bio2mar platform (http://bio2mar.obs-banyuls.fr) for technical assistance and access to Atlantic Islands. Arch. Environ. Contam. Toxicol. 70 (2), 265–288. https://doi.org/10.1007/s00244-015-0227-7.

Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S., Thomas, T. 2013. The seaweed holobiont: understanding seaweed-bacteria interactions. FEMS Microbiol. Rev. 37 (3), 462–476. https://doi.org/10.1093/femsre/fut056.

Eng, R.H.K., Padberg, F.T., Smith, S.M., Tan, E.N., Cherubin, C.E., 1991. Bactericidal effects of antibiotics. Antimicrob. Agents Chemother. 35 (9), 1824–1828. https://doi.org/10.1128/AAC.35.9.1824.

Fent, K., Zenker, A., Rapp, M., 2010. Widespread occurrence of estrogenic UV filters in wastewater, in surface waters, and in the environment in Switzerland. Environ. Pollut. 158 (5), 1817–1823. https://doi.org/10.1016/j.envpol.2009.11.005.

Feng, K., Zeng, A., Rapp, M., 2010. Widespread occurrence of estrogenic UV filters in ecosystems in Switzerland. Environ. Pollut. 158 (5), 1817–1823. https://doi.org/10.1016/j.envpol.2009.11.005.

Gackowska, A., Studziński, W., Kudlek, E., Dudziak, M., Gaca, J. 2018. Estimation of physicochemical properties of 2-ethylhexyl-4-methoxycinnamate (EHMC) degradation products and their toxicological evaluation. Environ. Sci. Pollut. Res., 1–13 https://doi.org/10.1007/s11356-018-1796-6.

Gago-Ferrero, P., Alonso, M.B., Bertozzi, C.P., Marigo, J., Inge, E.R., Azevedo, A., Isaac, E., Selva, B., Greci, L., Baschong, W., 2010. Assessment of the toxicity of sunscreens to the freshwater caddis Fly *Pseudoperla marginalis* and the benthic crustacean *Daphnia magna*. Environ. Sci. Technol. 44 (12), 4882–4889. https://doi.org/10.1021/es9031009.

Giraldo, A., 2017. Ecotoxicological Evaluation of the UV Filters Ethylhexyl Dimethyl P-Aminobenzoic Acid and Octocrylene Using Marine Organisms *Mytilus Galloprovincialis* and *Sericostoma Vittatum*. Environ. Sci. Technol. 51 (3), 1679–1686. https://doi.org/10.1021/acs.est.6b03056.

Gonzalez-Pastor, J.E., Hobbs, E.C., Losick, R., 2003. Cannibalism by sporulating bacteria. Cell (80) 301 (July), 510–515. https://doi.org/10.1021/acs.est.6b03056.

He, T., Tsui, M.M.P., Murphy, M.B., 2019. Comparative toxicities of four benzophenone ultraviolet filters to the two life stages of two coral species. Sci. Total Environ. 651 (10), pp. 5665–5677. https://doi.org/10.1016/j.scitotenv.2018.07.046.

Hengge, R. 2011. Phage-dependent gene regulation in *Escherichia Coli*. Am. Soc. Microbiol. (4), https://doi.org/10.1128/ecosal.01594-09.

Her, T., Tsui, M.M.P., Murphy, M.B., 2019. Comparative toxicities of four benzophenone ultraviolet filters to the two life stages of two coral species. Sci. Total Environ. 651 (10), pp. 5665–5677. https://doi.org/10.1016/j.scitotenv.2018.07.046.

Jentzsch, F., Olsson, O., Westphal, J., Reich, M., Leder, C., Kümmerer, K., 2016. Ecotoxicity of two organic UV filters to the freshwater caddis Fly *Sericostoma Vittatum*. Environ. Pollut. 228, 370–377. https://doi.org/10.1016/j.envpol.2017.05.021.

Kameda, Y., Kimura, K., Miyazaki, M., 2011. Occurrence and environmental distribution of 5 UV filters in marine environments of Okinawa island. Environ. Pollut. 159 (6), 1570–1576. https://doi.org/10.1016/j.envpol.2011.02.055.

Kim, H.J., Lee, E., Lee, M., Ahn, S., Kim, J., Liu, J., Jin, S.H., Ha, J., Bae, H., Lee, T.R., et al., 2018. Photophosphodiesterase 4B plays a role in Benzophenone-3-induced Phototoxicity in Normal human keratinocytes. Toxicol. Appl. Pharmacol. 328 (November 2017), 174–181. https://doi.org/10.1016/j.taap.2017.11.021.
