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Note

Exposure of key marine species to sunscreens: Changing ecotoxicity as a possible indirect effect of global warming

Paolo Fastelli, Monia Renzi*

Bioscience Research Center, via Aurelia Vecchia, 32, 58015 Orbetello, Italy

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ABSTRACT

Sunscreens can induce ecotoxicological effects and may cause significant impacts in the aquatic ecosystem. In spite of that, ecotoxicological responses of key marine species to sunscreens are scarcely studied in Mediterranean ecosystems, and literature data are lacking. Furthermore, changes in water salinity induced by global warming could significantly affect the ecotoxicological responses of marine species exposed to sunscreens. This research focuses on the evaluation of ecotoxicological responses of *Phaeodactylum tricornutum* (algae), *Corophium orientalis* (macroinvertebrate), and *Paracentrotus lividus* (echinoderms) exposed to sunscreens, which include both chemical- and physical-based. This study, also, analyzes the changes in ecotoxicological responses of the tested species linked to increase in salinity. Results showed that salinity stress significantly increases the toxicity of sunscreens on the tested marine species. Physical-based sunscreens resulted in more toxicity at higher salinity than chemical-based ones toward *C. orientalis* and *P. tricornutum*. This study evidenced that risk classifications of sunscreens recorded under standard salinity conditions could be significantly different from that recorded in the natural environment under salinity stress. The collection of a complete dataset on the ecotoxicological effects of sunscreens on marine species tested under salinity stress could be useful to correctly weigh risks for the marine environment under possible future ecological changing scenarios following the global changing driver.

1. Short note

Recent studies have evidenced how sunscreen released from human skin during bathing affects the health and preservation of the ecosystem (Danovaro et al., 2008; Tovar- Sánchez et al., 2013; Sánchez-Quiles and Tovar-Sánchez, 2015). There are two different types of sunscreen filters: chemical-based, which work by changing UVA/UVB rays into heat, and physical-based, which deflect and scatter UVA/UVB rays. Physical-based filters more widely used in sunscreens are represented by metal oxide in their nanoform, in particular, by nano-ZnO and nano-TiO_2; their use in cosmetics is allowed by law. Metal oxides in their nanoform are widely used in pharmaceutical and personal care products (WWC, 2013; Hossain et al., 2014). In particular, they are largely used as UVA/UVB filters in organic-made labeled sunscreens, for which any synthetic-made or petroleum-based chemicals are allowed. Nanoparticles are a heterogeneous group of different solid substances (i.e., functionalized fullerenes, polyethylene glycol, zeolites, ceramics, TiO_2, ZnO; Nowack and Bucheli, 2007; Kumar et al., 2014) characterized with a size range of 1–100 nm (Moore, 2006). Recent studies on the ecotoxicological effects of nanoparticles both as pure substances and as dissolved mixtures evidenced some worrisome aspects related to their environmental effects (Pettitt and Lead, 2013; Wang et al., 2014; Renzi and Blaskovic, in press). The nanoparticles were ecotoxic to the aquatic biota (Ducrotoy and Mazik, 2011; Mukherjee and Acharya, 2018); their dimension resulted in a functional trait able to significantly affect the toxicity of nanoparticles (Sun et al., 2009; Pettitt and Lead, 2013). For the reasons stated above, environmental risks associated with environmental diffusion of both chemical- and physical-based sunscreens could be very different as also reported by a recent study evidencing that nanoparticles in sunscreen may increase environmental concentrations of reactive oxygen, albeit to a limited extent, which can influence the transformation of dissolved substances and potentially affect ecosystem processes (Hanigan et al., 2018).

Summer tourism has a significant impact on marine ecosystems along the Mediterranean coastline; this particularly concerns countries like Italy, where approximately 8000 km of coastline is open to bathing during summertime (Renzi et al., 2012). Sánchez-Quiles and Tovar-Sánchez (2015) reported sunscreens as a new environmental risk associated with coastal tourism. Recent studies have measured sunscreens at concentrations ranging within 0.01 µL/L (Bratkovics et al.,

* Corresponding author.
E-mail address: monia.renzi@bsrc.it (M. Renzi).

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2015)–1395 μL/L (Downs et al., 2016) in shallow water. Furthermore, recent literature evidenced that global changes driven by global warming will not only affect temperatures but also determine other indirect effects such as water acidification (Dupont et al., 2010) that are able to affect marine species and rocky subtidal communities (Asnaghi et al., 2013; Asnaghi et al., 2014). Global warming may also induce changes in salinity that could affect marine ecosystems in both directions at a local scale due to phenomena such as ice-melting, changes in rain inputs, and evaporation (Durack and Wijffels, 2010). Effects of water acidification on ecotoxicological responses of marine species exposed to chemicals under both controlled pH and temperature patterns are evidenced in the scientific literature (Serra-Compte et al., 2018). Nevertheless, salinity changes could also affect ecotoxicological responses with regard to the bioavailability of chemicals, especially metal oxides (Chou et al., 2018). Furthermore, ecotoxicological responses of species exposed to chemicals under salinity stress could be significantly different from responses under optimal osmotic conditions.

This study aims to evaluate ecotoxicological responses of three key marine species at different trophic levels that are exposed to different sunscreens under standard salinity conditions. The selected species are considered of particular ecological significance by ISPRA (2011) according to their relative relevance reported and scores of 17 (*Paracentrotus lividus*), 21 (*Phaeodactylum tricornutum*), and 23 (*Corophium orientalis*) ranging within a maximum value of 23.5.

Furthermore, this research evaluates ecotoxicological responses on the tested species following the exposure to the same solutions under salinity stress.

Sunscreens based on two different formulas were tested: a chemical-based UV filter and an organic formula based on metal oxides added in their nanoform as a UV filter. Table 1 shows the general data of the tested sunscreens (i.e., SPF, filter formula, etc.). A starting sunscreen/water ratio of 100 μL/L was selected for geometric tested dilutions as reported in literature on coral reef environments (Danovaro et al., 2008). Natural marine water was filtered using a 0.45 μm mesh, and the filtered water used to perform dilutions of both sunscreens and negative controls. Tests were performed under two different salinity conditions: standard optimal salinity (3.5%, LS) and salinity osmotic stress (HS, 4.0%). The filtered natural marine water was opportunely corrected to the desired salinity by the addition of Milli-Q distilled water or NaCl concentrated saline solution before exposure experiments. Ecotoxicological tests were performed on *P. tricornutum* (algae), *C. orientalis* (Crustaceans), and *P. lividus* (Echinodermata) following standard methods (Table 2). Standard salinity condition 3.5% was fixed according to the testing methods as reported in Table 2. BsRC (certified laboratory ISO 9001:2015) applies a severe control procedure under the guidelines of the UNI EN ISO 17025 to ensure quality of data (1715L, Accredia). Quality control and quality assurance were performed as described by the testing methods. Positive controls were performed by direct exposure of the tested species to standard toxicants as detailed in Table 2. Experimental blanks were performed with natural marine water at both tested salinities to evaluate not only possible laboratory bias (standard optimal salinity) but also effects induced by the salinity osmotic stress. Recorded data were within the acceptability of tests (effects < 10% in LS). Results were normalized with regard to their negative controls according to salinity. Univariate and multivariate analyses were performed using Prism Graphpad software, $p < 0.01$ were considered statistically significant. Two-way ANOVA tests were performed on each species to evaluate interactive effects among salinity and sunscreen type stressors.

The results obtained under standard salinity conditions (3.5%) are summarized in Fig. 1. EC20 was represented (see figure for more details on EC meanings). Under standard salinity conditions, EC50 resulted in higher values than the maximum tested concentration (100 μL/L) for *P. tricornutum* (1-2SC), *C. orientalis* (2SC), and *P. lividus* (1SC). *C. orientalis* exposed to chemical-based sunscreen (1SC) showed an EC50 of 96 μL/L, while *P. lividus* exposed to physical-based sunscreen showed an EC50 of 14 μL/L. On the basis of EC50 and EC20 values recorded, chemical-based sunscreen resulted in significantly (*t*-test, $p < 0.01$) lower toxicity than physical-based ones concerning crustaceans. On the contrary, algae are highly and significantly affected (*t*-test, $p < 0.01$) by chemical-based sunscreens (1SC), while effects on the larval stages of *P. lividus* were similar in both cases.

Fig. 2 shows the EC50 effects observed for each species under standard optimal salinity conditions and under high salinity stressors. The reported values showed significant difference (*t*-test, $p < 0.01$) between the tested sunscreen types. An increased toxicity of both sunscreen formulas under salinity stress was recorded. Under salinity stress, EC50 was measurable in all tested marine species. EC50 levels were similar between sunscreen formulas concerning *C. orientalis* (82 μL/L in 1SC and 87 μL/L in 2SC), while EC50 levels were notably lower in physical-based sunscreen for algae and echinoderms (48 vs. 9.9 μL/L in *P. tricornutum* and 71.0 vs. 16.9 μL/L in *P. lividus,* respectively, in 1SC and 2SC). These results evidence the higher toxicity of physical-based sunscreen for algae and echinoderms than for crustaceans. Two-way ANOVA results are shown in Table 3. A significant interactive effect between sunscreen types and salinity stress is recorded for all tested species.

Danovaro et al. (2008) evidenced that exposure of marine ecosystems to sunscreen at doses comparable to those tested in this study could represent a threat for tropical ecosystems. This study confirmed that the tested levels could represent a risk for Mediterranean key species such as unicellular algae, echinoderms, and crustaceans. In Europe, ecotoxicological risks for the aquatic environments due to personal care products (i.e., sunscreens), should be evaluated under standard laboratory conditions before commercialization following the REACH framework Directive. Since May 2018, REACH is applied to any manufacturing product commercializing in Europe > 1 tonne/year. Nevertheless, commerce under this weight threshold is not regulated. According to REACH, ecotoxicity of personal care products to aquatic environments in European countries is assessed according to standard testing guidelines recommended by the Organisation for Economic Cooperation and Development (OECD). Results obtained according to standard OECD experimental conditions are associated with the commercialized product as descriptive of the possible risks for the aquatic environment (Hund-Rinke et al., 2018). Tests are performed on freshwater species (under low salinity levels), and testing on key marine species is not regulated and hence occasionally performed. Future

Table 1
General data on tested sunscreen creams. The tested sunscreens belonged to two different Italian commercial trademarks. Sun protection factor (SPF) was 15 for both tested sunscreens. Composition of the active ingredients of the commercial formula of sunscreen is listed. SPF measures the fraction of sunburn-producing UV rays that reach the skin. Notes: 1SC is a large market trademark based on chemical sunscreen (works by converting UVA/UVB rays into heat); 2SC comes from an organic labeled and certified Italian trademark based on physical sunscreen (works by deflection and scattering of UVA/UVB rays).

	Sunscreen type	Sunscreen[a]
1SC	Chemical	Bis-Ethylhexyloxyphenol methoxyphenyl triazine; Butyl methoxydibenzoylmethane; Ethylhexyl methoxycinnamate; Ethylhexyl salicylate
2SC	Physical	n-TiO_2; n-ZnO

[a] Alphabetical order.

Table 2
Ecotoxicological tests performed on marine species, general information, and optimal standard conditions.

	C. orientalis	*P. tricornutum*	*P. lividus*
Taxonomic group	Crustacea	Algae	Echinodermata
Testing method	ISO 16712:2005(E)	ISO 10253:2016(E)	EPA 600/R-95-136/Section 15
Endpoint	Lethality after 96 h	Growth inhibition after 72 h	Embryotoxicity after 72 h
Type of test	Acute	Acute	Acute
Salinity range (%)	0–3.6	3.0 ± 0.5	3.5 ± 0.1
Positive test toxicant	$CdCl_2$	$K_2Cr_2O_7$	$Cu(NO_3)_2*3H_2O$
EC50 range	1.56–4.38 mg Cd/L	16.21 ± 1.72 mg/L	22.6–68.34 µg/L

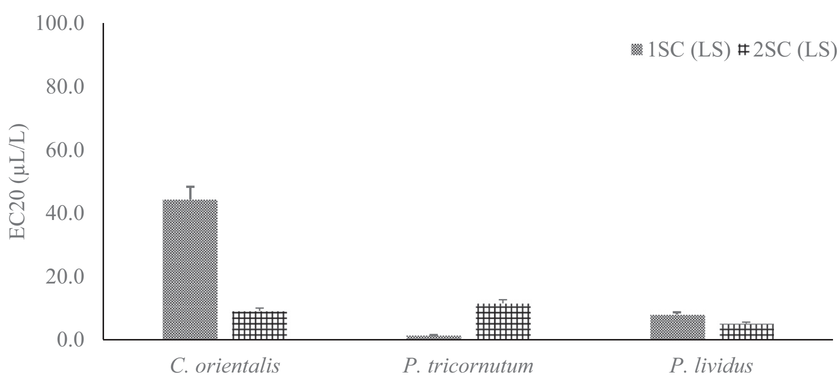

Fig. 1. EC20 recorded under standard salinity conditions (3.5%, LS) in tested species. Lower EC20 values mean higher toxicity. Standard deviations are evidenced. EC20 is the concentration of a test substance that results in a 20% reduction in a tested endpoint (i.e., survival rate for crustaceans, growth rate for algae, and abnormal embryos for echinoderms) relative to the control. 1SC = chemical-based sunscreen type; 2SC = physical-based sunscreen type. Significant differences (*t*-test, $p < 0.01$) are reported for *C. orientalis* and *P. tricornutum*.

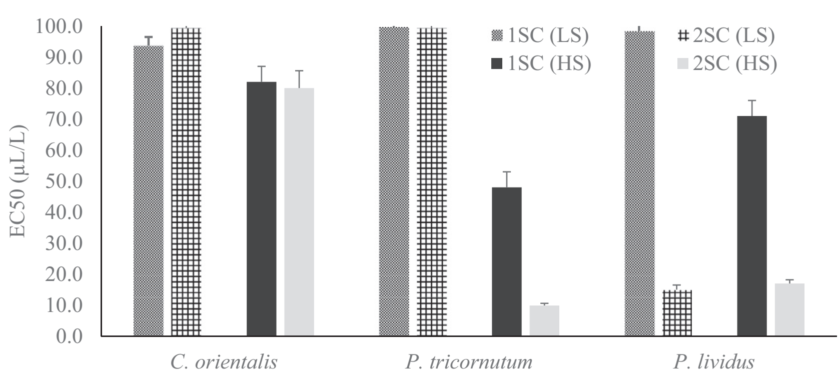

Fig. 2. EC50 recorded under different salinity conditions. EC50 is the concentration of a test substance that results in a 50% reduction in a tested endpoint (i.e., survival rate for crustaceans, growth rate for algae, and abnormal embryos for echinoderms) relative to the control. Standard optimal salinity (3.5%, LS) and high salinity (4.0%, HS) were tested. The represented differences are significant ($p < 0.001$). Under osmotic stress conditions (HS), negative controls showed effects that were always lower than 15%. Represented results are corrected according to the effect recorded on negative controls. 1SC = chemical-based sunscreen type; 2SC = physical-based sunscreen type.

Table 3
Two-way ANOVA performed on effects recorded (maximum exposure dose). Notes: Source of variation Su = Sunscreen types (Df 1); Sal = salinity stress (Df 1); Su x Sal = interactive effect (Df 1), Residual (Df 8); Significant *** = $p < 0.0001$.

Source of variation	Sum-of-squares	Mean square	F	% of total variation	p value summary
C. orientalis					
Su	1889	1889	2072	23.66	***
Sal	4198	4198	4606	52.59	***
Su X Sal	1889	1889	2072	23.66	***
Residual	7.292	0.9115			
P. tricornutum					
Su	340.4	340.4	442.8	4.02	***
Sal	7089	7089	9222	83.63	***
Su X Sal	1041	1041	1354	12.28	***
Residual	6.150	0.7688			
P. lividus					
Su	2179	2179	2060	34.41	***
Sal	2434	2434	2302	38.44	***
Su X Sal	1711	1711	1618	27.02	***
Residual	8.460	1.057			

implementation of European guidelines should improve the required ecotoxicological assessment to correctly evaluate the risk of chemicals to marine environments before the commercialization of chemicals, the use of which implies the direct release of chemical substances into marine environments as in the case of sunscreens. Our research points out that chemical-based and physical-based sunscreens could exert different ecotoxicological responses on the tested species, suggesting that sensitivity toward nanoparticles in personal care products could be very different in the considered marine taxa. Further specific studies on these aspects are needed to better understand the dynamics and associated risks for the marine environment. EC50 recorded in the tested species were found to be similar concerning standard salinity conditions, whereas under osmotic stress, toxicity on crustaceans and algae was amplified. With regard to toxicity of nanoparticles, they have significant structure, shape, and size in aquatic environments because of aggregation, solubilization, or adsorption (Handy et al., 2008) determining different toxicities on the biota. Effects caused by the exposure to complex mixtures are significantly different from effects caused by the exposure to single substances, although most of these compounds are present at low concentrations (Schwarzenbach et al., 2006).

Our results evidenced that salinity stress could be effective on changing ecotoxicological responses as reported in the literature for temperature (Prato et al., 2008) and pH (Dupont et al., 2010; Asnaghi et al., 2013; Asnaghi et al., 2014). Even though the salinity level is indirectly affected by global warming, a variation in salinity of 0.5% (3.5% vs. 4.0%) may affect ecotoxicological responses of marine species. Further studies in this field are needed. Nevertheless, these results suggest that global warming could affect ecotoxicological risk assessment performed under standard test conditions required in European countries on personal care products. Furthermore, a significant interaction between sunscreen types and salinity stress is reported in this study for each of the three tested species, and recorded trends cannot be generalized, but data should be collected case-by-case to evaluate effects on ecotoxicity.

Further studies on the effect of the level of aggregation among UV-filter nanoparticles and the organic matrix in sunscreens will be performed by light scattering and FESEM approaches to evaluate the interferences of this aspect on observed ecotoxicity. In fact, recent literature evidenced that free hydroxyl radicals produced in aqueous suspensions by nano-TiO_2 and nano-ZnO varied with aggregate structure and size (Jassby et al., 2012), which both represent factors that significantly affect ecotoxicological responses to free radical-induced stress. The behavior of nanoparticles in water dispersions, such as the level of aggregation recorded by dynamic light scattering, could be significantly affected by adsorption of organic substances onto TiO_2 nanoparticle surface (Almusallam et al., 2012).

Recent studies have evidenced that UV filters present in the formulation of sunscreens are detected in nearshore water and are concentrated in the surface microlayer ranging within 6.9–37.6 mg/L for TiO_2 and 1.0–3.3 mg/L for ZnO (Tovar- Sánchez et al., 2013). Because of the ecotoxicity reason, sunscreens, among other personal care products used by humans, may represent a critical concern for the coastal marine ecosystem, as their use implies direct dispersion in the environment caused by the contact of human skin with marine water during bathing.

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