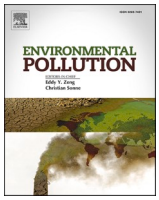
Environmental Pollution 351 (2024) 124093

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol



Inorganic UV filter-based sunscreens labelled as eco-friendly threaten sea urchin populations☆

F. Marcellini [a,b], S. Varrella [a,b], M. Ghilardi [a,1], G. Barucca [c], A. Giorgetti [a], R. Danovaro [a,b], C. Corinaldesi [b,c,*]

[a] Department of Life and Environmental Sciences, Polytechnic University of Marche, Via Brecce Bianche, 60131, Ancona, Italy
[b] National Biodiversity Future Centre, Italy
[c] Department of Materials, Environmental Sciences and Urban Planning, Polytechnic University of Marche, Via Brecce Bianche, 60131, Ancona, Italy

ARTICLE INFO

Keywords:
Eco-friendly sunscreens
Paracentrotus lividus
Inorganic UV filters
Zinc oxide
Titanium dioxide

ABSTRACT

Although the negative effects of inorganic UV filters have been documented on several marine organisms, sunscreen products containing such filters are available in the market and proposed as eco-friendly substitutes for harmful, and already banned, organic UV filters (e.g. octinoxate and oxybenzone). In the present study, we investigated the effects of four sunscreen products, labelled by cosmetic companies as "eco-friendly", on the early developmental stages of the sea urchin *Paracentrotus lividus*, a keystone species occurring in vulnerable coastal habitats. Among sunscreens tested, those containing ZnO and TiO_2 or their mix caused severe impacts on sea urchin embryos. We show that inorganic UV filters were incorporated by larvae during their development and, despite the activation of defence strategies (e.g. phagocytosis by coelomocytes), generated anomalies such as skeletal malformations and tissue necrosis. Conversely, the sunscreen product containing only new-generation organic UV filters (e.g. methylene bis-benzotriazolyl tetramethyl, ethylhexyl triazone, butylphenol diethylamino hydroxybenzoyl hexyl benzoate) did not affect sea urchins, thus resulting actually eco-compatible. Our findings expand information on the impact of inorganic UV filters on marine life, corroborate the need to improve the eco-friendliness assessment of sunscreen products and warn of the risk of bioaccumulation and potential biomagnification of inorganic UV filters along the marine food chain.

1. Introduction

Coastal tourism, and related recreational activities, determine a massive release of sunscreens into the marine environment (Danovaro et al., 2008; Tovar-Sánchez et al., 2019; Labille et al., 2020).

The sunscreen market, one of the fastest-growing segments of the personal care industry (annual growth rate of 2.15%; Naik et al., 2022) is expected to reach 10.4 billion USD by 2024.

Sunscreen products typically contain organic (e.g. cinnamates, camphor derivatives, benzophenones) and/or inorganic UV (e.g. TiO_2 and ZnO) filters as well as other ingredients (e.g. preservatives, adjuvants, moisturizers, antioxidants), which have been widely reported to impact marine organisms. The threats due to sunscreen dispersal in the marine environment are pervasive, from prokaryotes to large animals, and act in multiple ways, from the molecular (e.g. gene expression, DNA damage), to cellular (e.g. production of reactive oxygen species) and community/ecosystem levels (e.g. mortality of organisms, behavioural alteration; Lozano et al., 2020 and references therein). Organic UV filters due to their persistence and capability to bioaccumulate within marine organism tissues and organs may represent a risk also for the transfer and biomagnification along the food web (Lozano et al., 2020; Board National Academies of Sciences, Engineering, and Medicine, 2022).

The marketing and use of some organic UV filters (e.g. oxybenzone, octinoxate) have been banned in several countries around the world because they are considered a risk to marine ecosystems (Miller et al., 2021). However, in the last years, the combination of organic and inorganic compounds has constantly increased due to the broader UV

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

* Corresponding author. Department of Materials, Environmental Sciences and Urban Planning, Polytechnic University of Marche, via Brecce Bianche, 60131, Ancona, Italy.
E-mail address: c.corinaldesi@univpm.it (C. Corinaldesi).
[1] Present address: PSL Université Paris, CRIOBE, EPHE-CNRS-UPVD, 58 Avenue Paul Alduy, 66000 Perpignan, France.

https://doi.org/10.1016/j.envpol.2024.124093
Received 24 January 2024; Received in revised form 12 April 2024; Accepted 30 April 2024
Available online 2 May 2024
0269-7491/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

spectrum of protection (Labille et al., 2020). In addition, inorganic UV filters have been suggested to be eco-friendly alternatives to organic UV filters (Hojerová et al., 2011; Blasco et al., 2020). Titanium dioxide (TiO_2) and zinc oxide (ZnO), in their nano form, are inorganic UV filters used in sunscreens (Smijs and Pavel, 2011), especially as substitutes for synthetic- or petroleum-based filters in products defined as "green" or "eco-labelled" products (Fastelli and Renzi, 2019).

However, recent studies on nanoparticles, including ZnO and TiO_2, have reported multiple adverse effects of these compounds, tested as single ingredients or as a mixture of UV filters, on a wide range of marine organisms (Sendra et al., 2017; Corinaldesi et al., 2018; Tovar-Sánchez et al., 2019; Catalano et al., 2020; Cunningham et al., 2020; Roma et al., 2020; Tovar-Sánchez et al., 2020; Miller et al., 2021). Other research has also documented the negative effects of non-nano inorganic UV filters on marine species (Oliviero et al., 2017; Lozano et al., 2020). Furthermore, considering the particle aggregation and fragmentation as well as other biological, physical and chemical processes to which these filters can be subject once released into seawater (Lead et al., 2018; Yuan et al., 2022), the relationship between size and negative effect in the natural environment remains not fully understood.

The increasing interest in human and environmental health has boosted the personal care industry to create a strategy that could motivate many consumers to purchase organic or natural personal care products (Amberg and Fogarassy, 2019). Cosmetic companies have attempted to respond to the growing environmental concerns of consumers with the introduction of a large variety of eco-friendly cosmetics (Cosmetic Europe annual report, 2020), with an increase in Europe of more than 7% in Ecolabel products in recent years alone (Ecolabel Report, 2021). However, currently, there are no universally recognized regulatory standards that establish criteria for the use of the eco-compatibility claim in sunscreen products nor universal certification of ecological quality (Corinaldesi et al., 2017). Available certifications for eco-friendly sunscreens typically involve validation from reputable organizations that assess only some specific aspects of sustainability, packaging materials, biodegradability, or ingredient safety but most of them do not consider the effects on marine organisms in the laboratory or the natural environment (Adler and DeLeo, 2020; Pawlowski et al., 2023).

The present study aims to assess the impact of different brands of sunscreen products labelled as "eco-friendly, natural, green and reef safe", containing nano - and non-nanoparticle inorganic UV filters (TiO_2 and/or ZnO), and/or organic UV filters (e.g. octocrylene, butyl methoxydibenzoylmethane, homosalate, ethylhexyl salicylate) on the sea urchin *Paracentrotus lividus*. *P. lividus* represents a key species that plays a fundamental ecological role in the Mediterranean Sea and the eastern Atlantic Ocean and is also considered a model organism for studying the impact of numerous contaminants (Falugi et al., 2012; Gambardella et al., 2021). This species is found in shallow coastal habitats, i.e. those most threatened by recreational activities and frequented by bathers (Boudouresque and Verlaque, 2020) and therefore potentially affected by contamination from sunscreens (Keller, 2023).

The objective of the study was also to compare the effects of inorganic UV filter-based sunscreens with those containing new-generation UV filters (e.g., ethylhexyl triazone, methylene bis-benzotriazolyl tetramethylbutylphenol, diethylamino hydroxybenzoyl hexyl benzoate; Varrella et al., 2022) reported as environmentally friendly (Corinaldesi et al., 2017), and with ZnO (as a single ingredient), which has been reported cause severe impacts on marine organisms, even stronger than TiO_2 (Schiavo et al., 2016; Corinaldesi et al., 2018; Yuan et al., 2023; Keller, 2023), to identify a scale of impact which could help the decision-making process in the development of effective eco-compatible sunscreens.

2. Materials and methods

2.1. Sunscreens product and zinc oxide nanoparticles

Four commercially available sunscreen products containing inorganic and/or organic UV filters and zinc oxide nanoparticles were selected.

The following ingredients and sunscreen products were tested:

Sunscreen 1 (SS1): labelled as eco-compatible and characterized by the presence of a new patented UV filter with TiO_2 non-nanoparticles and alumina, and other ingredients such as emulsifiers (i.e. sucrose stearate), along with other organic compounds such as *Simmondsia chinensis* oil and candelilla cera.

Sunscreen 2 (SS2): labelled as eco-compatible and containing organic new generation UV filters (i.e. ethylhexyl triazone, methylene bis-benzotriazolyl tetramethylbutylphenol; diethylamino hydroxybenzoyl hexyl benzoate), preservatives (i.e., potassium sorbate, sodium benzoate), and other constituents such as *Citrus aurantium dulcis* peel oil, propylene glycol and caprylyl glycol.

Sunscreen 3 (SS3): labelled as biodegradable and "reef safe" and characterized by TiO_2 and ZnO non-nanoparticles (6.4% and 6.0%, respectively), aluminium hydroxide ($Al(OH)_3$), antioxidants, and >85% of organic ingredients (i.e., aloe vera, glycerin, different seed oils).

Sunscreen 4 (SS4): defined as "safe sea" has been patented also for offering protection against jellyfish stings and contains TiO_2 nanoparticles (nano TiO_2) and organic UV filters (i.e. octocrylene, homosalate, ethylhexyl salicylate, butyl methoxydibenzoylmethane). It also contains silicones (dimethicone, cyclopentasiloxane), and emollients (dibutyl adipate, dicaprylyl ether).

Zinc oxide: purchased from Sigma-Aldrich (cod. 96479), characterized by uncoated particles of size ranging from 20 to 200 nm (nanoparticles with size 20–100 nm > 50% of the total particles), confirmed by Scanning Electron Microscopy (SEM). Due to the predominance of nanoparticles, we defined this compound as ZnO nanoparticles (nano ZnO).

The complete list of ingredients is available in Table S1 in the Supplementary Online Material.

The concentration used in our experimental systems (50 μL L^{-1}, Danovaro et al., 2008; Corinaldesi et al., 2017) is a conservative estimate of the sunscreen amount released during the tourist season in the coastal intertidal areas of the Mediterranean (Varrella et al., 2022). The Mediterranean Sea is a priority area to evaluate the impact of sunscreen products as its coasts are among the most important tourist destinations (Mejjad et al., 2022).

Nano ZnO was tested at two different concentrations (low: 6.3 mg L^{-1} and high: 12.5 mg L^{-1}). The low and high nano ZnO concentrations were defined according to the typical concentrations of inorganic UV filters used in sunscreen products. In particular, we used a lower concentration of inorganic UV filters equals to 6.3 mg L^{-1} (corresponding to 12 % of 50 μL L^{-1} of sunscreen products, i.e. the minimum concentration typically found in marketed sunscreen formulas; Corinaldesi et al., 2018), and a higher concentration equals to 12.5 mg L^{-1} (corresponding to 25% of 50 μL L^{-1} of sunscreen product; i.e. the maximum concentration allowed in cosmetic preparations by the EU Directive (Commission Regulation (EU) 2016/621, amending Annex VI to Regulation (EC) No. 1223/2009 of the European Parliament) and the US Food and Drugs Administration, FDA (Document 84 FR 6204, Sunscreen Drug Products for Over-the- Counter Human Use).

These values fall within the range of values of the same inorganic UV filters tested in previous works (Libralato et al., 2013; Sendra et al., 2017; Khosravi-Katuli et al., 2018).

Sunscreen products were also selected for claims reported as "ecological, natural, organic, and reef safe". The names assigned to the sunscreens tested in the experiments (i.e. SS1, SS2, SS3 and SS4) do not correspond to the real names of the products.

2.2. Collection of P. lividus individuals and gametes

Adult specimens of *P. lividus* were collected during the breeding season by SCUBA divers along the shore of the Central Adriatic Sea (43°37′11.29″N 13°31′52.9″E, Mediterranean Sea, 11 °C, Salinity 38.3, pH 8.2).

P. lividus specimens were immediately transported to the laboratory, where were kept in aquaria with filtered seawater (onto 0.22 μm pore size filters, Aisimo®) for at least 1 week, at 14 °C and *in situ* salinity and pH conditions.

The gamete spawning was obtained according to Amemiya (1996) from 3 males and 3 females, through an oral injection of 0.5M acetylcholine chloride diluted (1:1000) with autoclaved and ultra-filtered seawater (using 0.02 μm pore size Anotop® syringe filters, Whatman, Springfield Mill, UK). According to Falugi et al. (2008).

The eggs were released in filtered seawater and the sperm was collected directly from the genital pores and preserved at 4 °C. One hundred μL of sperm diluted with ultra-filtered seawater (1:10) were added to 130 μL of ultra-filtered seawater containing 3000 eggs (counted using a microscope, objective 10×, 100 × magnification, Zeiss Axioskop). The fertilization rate was evaluated by counting 300 eggs using a light microscope (objective 10×, 100x magnification, Zeiss Axioskop). Batches of eggs that showed at least a 93% fertilization rate were used for testing the effects of sunscreen products and nano ZnO.

2.3. Exposure of P. lividus embryos to sunscreen products and ZnO nanoparticles

Our experiments were carried out under ISO-validated tests and Falugi et al. (2008). Seventy-two sterile containers with 100 mL of filtered seawater and a mix of female and male gametes (230 μL) were maintained at 18 °C according to Giudice (1986). Four sunscreens (at the final concentration of 50 μL L^{-1}) and ZnO nanoparticles (at two different concentrations: 6.3 mg L^{-1} and 12.5 mg L^{-1}) were added to containers (three replicates for each treatment with sunscreen and nano ZnO) immediately after the addition of the female and male gametes (Falugi et al., 2008), and compared with the controls (three untreated replicates). Sub-samples were collected from each container after 20 min of the addition of the different concentrations of sunscreens and ZnO nanoparticles, after fertilization (t_0), at the morula stage (after 3 h; t_3) and at the gastrula stage (after 24 h; t_{24}) from the beginning of the time-course experiment. The collected sub-samples were preserved with paraformaldehyde (4%, pH 7.4). Six hundred embryos were counted for each system and were morphologically characterized using a light microscope with objectives 10, 20 and 40x (100-400x magnification, Zeiss Axioskop). To obtain more information on the natural variability of the organisms, we avoided pseudo-replication using independent replicates.

2.4. Exposure of P. lividus larvae to sunscreen products and ZnO nanoparticles

Additional experiments to assess the impact of sunscreens and ZnO nanoparticles on larval development were performed. To develop four-armed larvae of *P. lividus* (Giudice, 1986; Gambardella et al., 2013) four sterilised glass containers were filled with 350 mL of filtered seawater, then 1 mL of concentrated eggs and 350 μL of diluted sperm (1:100 in filtered seawater) were added to seawater and incubated for 48 h at 18 °C in a thermostatically controlled room. Time-course experiments were carried out using 72 sterilised containers each containing approximately 250 *P. lividus* four-armed larvae (obtained as described above) in a total volume of 100 mL. The different sunscreen products and nano ZnO (at the two concentrations) were added to the glass containers as described in paragraph 2.3. Containers without the addition of sunscreen products and nano ZnO nanoparticles were used as controls. Sub-samples were collected from each container immediately after the addition of the sunscreens and nano ZnO (t_0), after 3 h (t_3) and 24 h (t_{24}) from the beginning of the time-course experiment. The collected sub-samples were preserved with paraformaldehyde (4%, pH 7.4). For each of them, we counted one hundred and fifty larvae, which were morphologically characterized using a light microscope with objectives 10 and 20x (100-200 × magnification, Zeiss Axioskop).

2.5. Morphological analysis of P lividus embryos and larvae exposed to sunscreens and ZnO nanoparticles

To assess the health of *P. lividus* embryos and larvae, we analyzed the morphology and synchronicity of their early developmental stages compared to the control (Corinaldesi et al., 2017; Gambardella et al., 2021) using a light microscope with objectives 10, 20 and 40x (100-400 × magnification, Zeiss Axioskop).

Morphological anomalies of embryos and larvae were described based on the classification reported by Gambardella et al. (2021).

Embryos were defined as normal if they showed a correct formation of the archenteron structure and migration of cells into the coelom, as described in previous studies (Corinaldesi et al., 2017).

Similarly, larvae at the pluteus stage were defined as normal if they showed a regular conical shape, four fully developed arms and complete skeletal rods similar in size to control larvae (Carballeira et al., 2012; Corinaldesi et al., 2017; Gambardella et al., 2021).

In the present study, four levels of severity of embryonic/larval anomalies (from 0 to 3; with level 0: absent damage corresponding to normal development, level 1: slight damage, level 2: moderate damage, and level 3: severe damage) both in the treated and control systems at t_{24h} (i.e. after 24 h from the beginning of the experiment) were considered.

We calculated the index of contaminant impact (ICI*) by modifying the formula developed by Carballeira et al. (2012), to also take into account the damage level of the embryos and larvae in the control systems because natural anomalies or alterations due to experimental manipulation, can occur without exposure to any treatment (i.e. in this case, the sunscreens). The ICI* was calculated as follows:

$$ICI^* = [(0 \times \Delta\% \text{ level } 0 + 1 \times \Delta\% \text{ level } 1 + 2 \times \Delta\% \text{ level } 2 + 3 \times \Delta\% \text{ level } 3)/100]$$

where: $\Delta\%$ level i = [(% level i) $_{treated}$ - (% level i) $_{control}$] for i = 0, 1, 2, 3.

Using algebraic operations, this formula is equivalent to:

$$ICI^* = (ICI_{treated} - ICI_{control})$$

The resulting index of contaminant impact (ICI*) can have values from −3 to 3: for values around 0 the frequency of anomalies for each degree of embryonic and larval alteration in the treatment is similar to the control; if the values are positive such a frequency in the treatment is higher than the control whereas when the values are negative it is lower.

2.6. Identification of Ti and Zn on P. lividus embryos with scanning electron microscopy (SEM) analyses

To investigate the presence of Ti and Zn in sea urchin larvae, after 24 h of exposure, the larvae exposed to SS3 were collected from paraformaldehyde-fixed samples and prepared for SEM analyses. Larvae were washed five times with filtered seawater and then stained with Rose Bengal dye (Sigma Aldrich, Germany). After 24 h, samples were carefully rinsed in filtered seawater, placed on a 0.2 μm polycarbonate filter, and subsequently dehydrated through a graded series of ethanol before critical point drying. The water was progressively replaced by increasing ethanol concentrations (10, 30, 50, 70, 80, 90, 95 and 100% vol distilled water: vol ethanol) for 3 h. Samples were subsequently stored in 100% ethanol overnight. The dried specimens were mounted on carbonium stubs and coated with gold through a sputter coater (Emitech K550) before observation under SEM (Zeiss SUPRA 40) Absorption of chemical elements was carried out with a microanalysis

system (Bruker Quantax Z200) at 20Kv.

2.7. Data analysis

To assess the differences (univariate tests) between treatments and controls, during the experiment permutational analyses of variance were used (PERMANOVA, McArdle et al., 2001; Anderson, 2005). The experimental design included two factors (time, and treatment). When significant differences were encountered ($p < 0.05$) post-hoc pairwise tests were also carried out. Statistical analyses were carried out using the routines included in the PRIMER 6+ software (Clarke and Gorley, 2006).

3. Results

3.1. Effect of sunscreen products and nano ZnO on sea urchin embryos

Among the sunscreens tested, the most significant and negative effects were observed in the systems treated with SS3 (based on non-nano TiO_2 and ZnO UV filters) and SS4 (based on nano TiO_2 and organic UV filters). SS4 determined a significant increase in the percentage of abnormal embryos compared to the control immediately (t_0) after the addition of the product ($p < 0.01$) and after 24 h (t_{24}) of exposure ($p < 0.001$) when 95.8% of abnormal embryos were observed (Fig. 1 A). Similarly, SS3 caused a higher percentage of anomalies than the control at the end of the experiment (t_{24}, $p < 0.001$), with 91.6% of abnormal embryos (Fig. 1 A). Conversely, SS2 (based on new generation organic

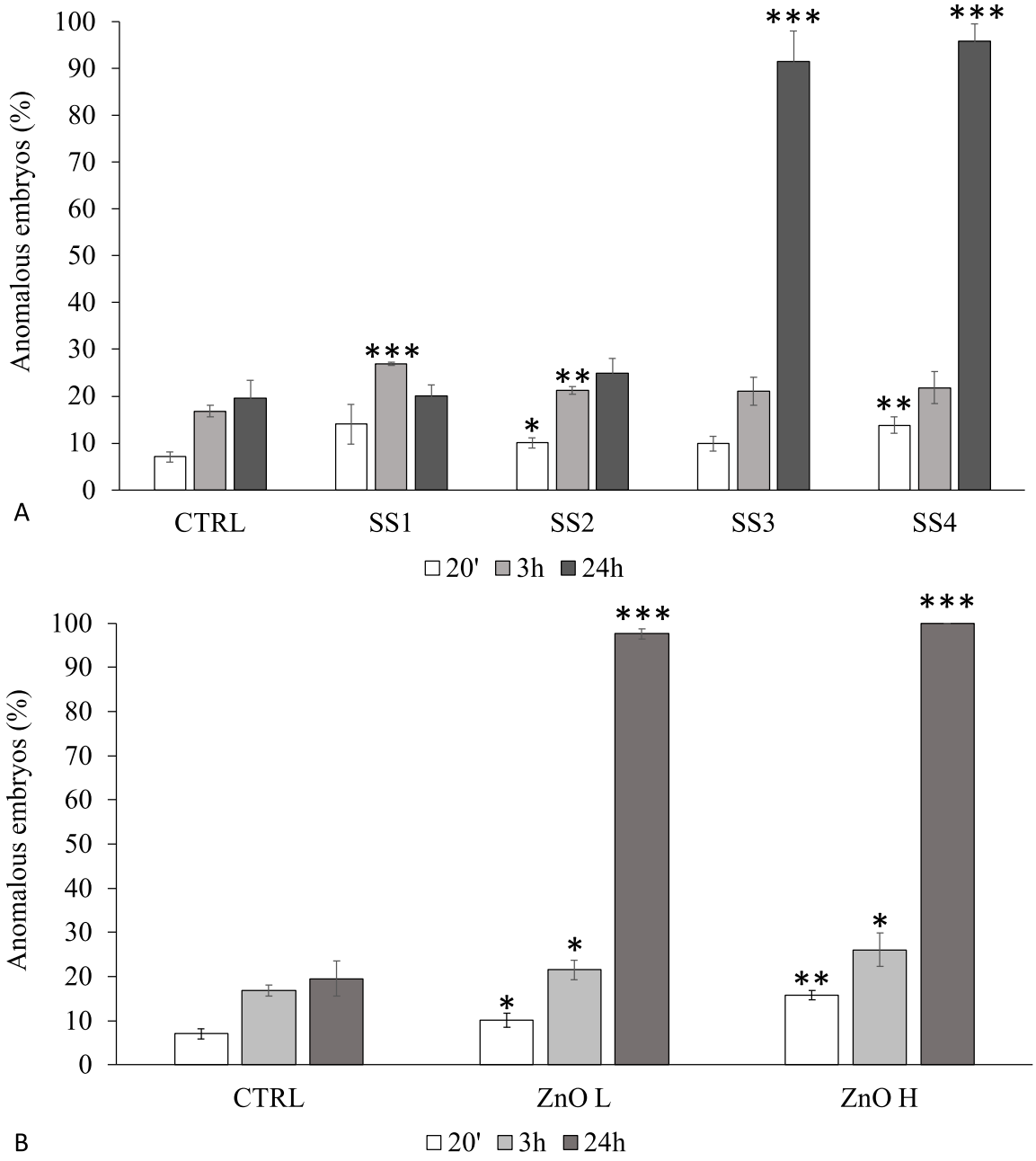


Fig. 1. *P lividus* embryonic anomalies. Percentage of embryonic anomalies at the three sampling times after exposure to (A) four different brands of sunscreens (SS1, SS2, SS3 and SS4) and (B) zinc oxide (ZnO) nanoparticles at low (L, 6.3 mg L^{-1}) and high (H, 12.5 mg L^{-1}) concentrations. Significant differences between treatments and the control systems are indicated with asterisks: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Error bars indicate the standard deviations ($n = 3$).

UV filters; Fig. 1 A) produced a significant increase in the anomalous embryos compared to the controls after 20 min (t_0, $p < 0.05$) and after 3 h (t_3, $p < 0.01$) from the addition of the sunscreen product, but no significant differences were observed after 24 h (Fig. 1 A).

An increase in the anomalous embryos treated with SS1 (based on non-nano TiO_2 UV filters) compared to the control was observed only at the morula stage (t_3, $p < 0.01$), but no significant differences were observed at the end of the experiment (t_{24}; Fig. 1 A).

The analyses conducted with nano ZnO at both concentrations tested (6.3 and 12.5 mg L^{-1}) caused the strongest negative effects in terms of embryo anomalies (Fig. 1 B), as highlighted by the percentage of anomalous embryos, which increased immediately after the addition of nano ZnO at both concentrations tested (ZnO L, $p < 0.05$; ZnO H, $p < 0.01$). These values, when compared to the controls, further increased significantly over time (t_3, $p < 0.05$; t_{24}, $p < 0.001$). After 24 h of exposure at the low and high nano ZnO concentrations, 97.6 and 100% of anomalies were observed, respectively (Fig. 1 B).

3.2. *Effect of sunscreen products and nano ZnO on sea urchin larvae*

Among the eco-compatible sunscreen products, the worst effects were observed in larvae exposed to SS4 (Fig. 2 A). A significant increase in larval anomalies (t_3, $p < 0.05$) was already visible after 3 h of exposure compared to the control, and these values increased over time, with 56.4 % of abnormal larvae after 24 h of exposure (t_{24}, $p < 0.01$). Similarly, SS3 induced a significant increase (t_3, $p < 0.05$) in the percentage of anomalous larvae after 3h of exposure (28.6%) and these values increased at the end of the experiment (t_{24}, $p < 0.01$) with 38.0% of the larval anomalies detected (Fig. 2 A).

The SS1 sunscreen product caused a significant increase in the percentage of abnormal larvae compared to the control only at the end of the experiment (t_{24}; 37.3%; $p < 0.001$) (Fig. 2A). Conversely, in systems treated with SS2, no significant differences ($p > 0.05$) were observed compared to the control in the percentage of abnormal larvae over time (Fig. 2A).

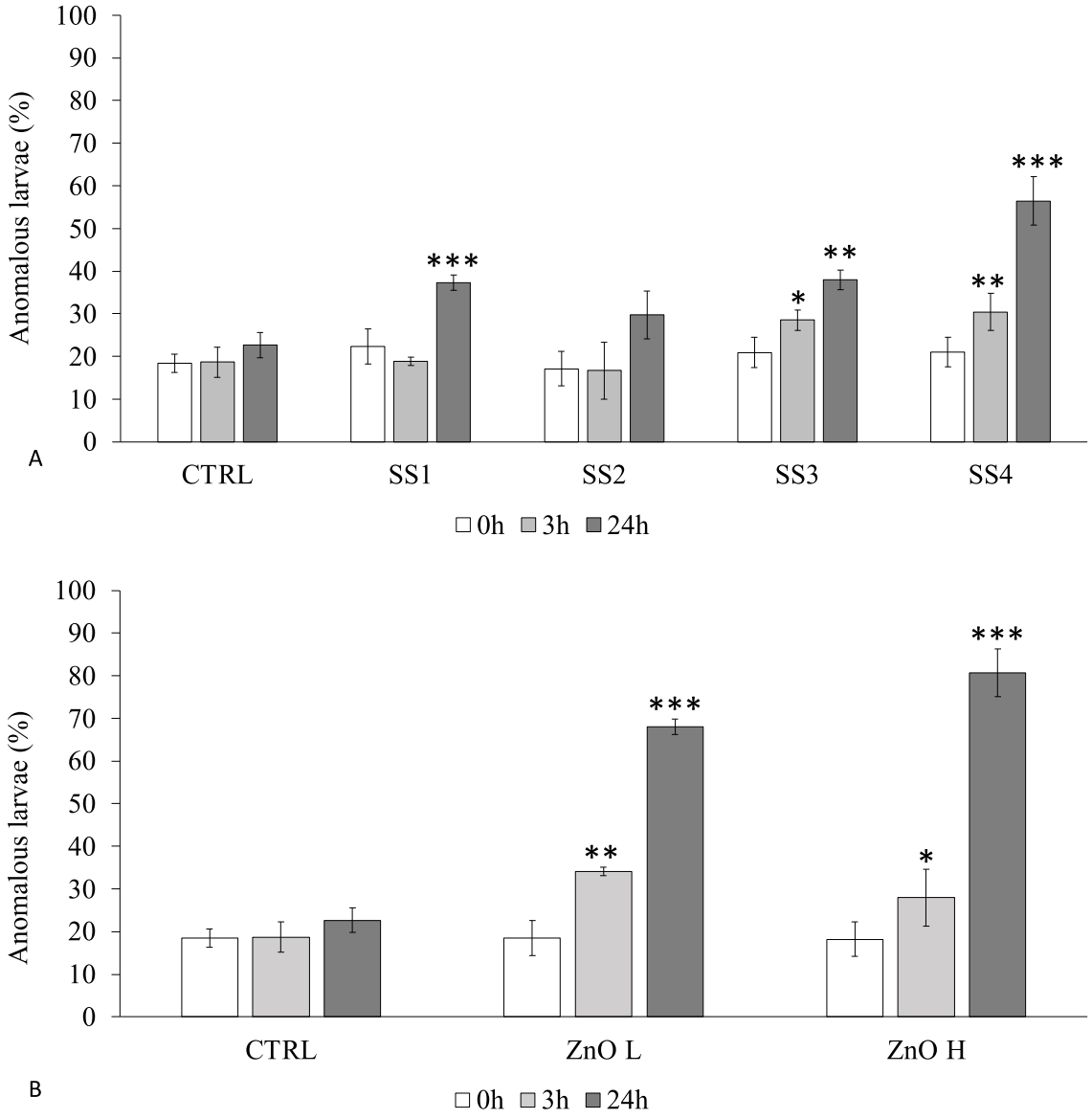


Fig. 2. *P lividus* larval anomalies. Percentage of larval anomalies at the three sampling times after exposure to (A) four different brands of sunscreens (SS1, SS2, SS3 and SS4) and (B) zinc oxide (ZnO) nanoparticles at low (L, 6.3 mg L^{-1}) and high (H, 12.5 mg L^{-1}) concentrations. Significant differences between treatments and the control systems are indicated with asterisks: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Error bars indicate the standard deviations (n = 3).

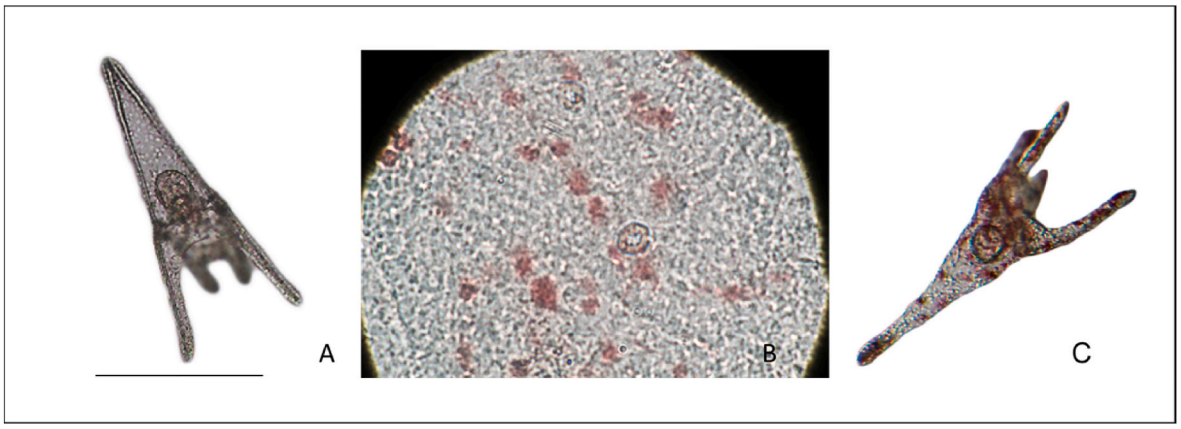


Fig. 3. *P. lividus* larva after 24 h of high Zinc oxide exposure. Unexposed larva (A) in comparison with larva treated with ZnO H characterized by the presence of the red amebocytes (C), details of the red amebocytes (B). Scale bars 200 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The addition of nano ZnO at both concentrations tested (low and high) induced a significant increase in the percentage of abnormal larvae (t_3, $p < 0.05$) compared to the control after 3 h of exposure, and these values increased after 24 h (t_{24}, $p < 0.001$), reaching 68.0 and 80.7% of anomalous larvae, respectively (Fig. 2 B).

The high-sensitivity SEM/Energy Dispersive X-ray (EDX) confirmed the presence of elemental zinc and titanium signals in the larval tissues exposed to SS3 (Table 1). In contrast, no noticeable peaks corresponding to Zn and Ti were observed in the control (Table 1, Fig. 7). A detailed chemical composition of EDS was shown in Table 1.

3.3. Morphological anomalies of P. lividus embryos and larvae and index of contamination impact

Overall, we found 10 types of embryonic anomalies (Table S2) and 13 types of larval anomalies (Table S3) in the treatments analyzed.

The types of anomalies observed in embryos and larvae exposed to the four sunscreen products and nano ZnO were similar, with a high frequency of anomalies of levels 2 and 3 (Figs. 4 and 5), which represent the most severe types of malformations in the early development of the sea urchin.

Among the sunscreen products investigated, SS4 determined the most negative effect on embryos and larvae, resulting in ICI* values equal to 1.47 and 0.69, respectively (Tables S4 and S5), with a higher frequency of embryos and larvae anomalies of levels 2 (78.1 and 38.4 % respectively) and 3 (16.8 and 7.6%, including forming exogastrulae and necrosis). SS3 caused a high impact on embryo development (ICI* = 1.46; Table S4) whereas determined a slight impact on larval development (ICI* = 0.24; Table S5). The results of the morphological analysis showed a higher percentage of delayed embryos after 24 h of exposure with SS3 and SS4 (61.8 and 78.1%, respectively) than the control (2.6%) and other treatments. While in the control the embryos were already at the prism stage (Fig. 6 A) or pre-pluteus (Fig. 6 B), the embryos exposed to SS3 and SS4 were largely at the early gastrula stage (Fig. 6 C).

The SS1 treatment, after 24 h of exposure, determined 23.3% of abnormal larvae of the level 2, and no impact on embryo development. This resulted in an ICI*value for larvae of 0.32 and an ICI*value for embryos of −0.08.

SS2 produced no impact on either embryos or larvae at the end of exposure, with values of ICI* (0.05 and 0.16 respectively) very close to 0 (control systems).

Exposure to nano ZnO, at both low and high concentrations, resulted in the highest index of contamination impact (ICI*) in *P. lividus* embryos and larvae (Tables S4 and S5). Especially after exposure to the high concentration of nano ZnO, we observed the highest percentage of abnormal embryos of level 3 (the most severe type of damage; 36.9%) (Fig. 4), which resulted in an ICI* of 1.58 (Table S4). The embryos exposed to the high concentration of nano ZnO at the gastrula stage showed a high percentage of embryonic malformations (15.4%), due to abnormal migration of primary mesenchyme cells, which failed to enter the coelomic cavity and were extruded forming exogastrulaes (Fig. 4 U, V). The low nano ZnO concentration determined an ICI* value of 1.52 (Table S4) due to the highest percentage of abnormal embryos within levels 2 (58.7 %) and 3 (28.3%). The most common embryonic malformations observed after 24 h of exposure at low and high nano ZnO-concentrations, were gastrulae with irregular shape due to anomalous migration of the primary mesenchyme (Fig. 4 S, T), exogastrulae (Fig. 4 U, V), embryos in an advanced stage of necrosis (Fig. 4 W), and a severe

Table 1
SEM/EDS data obtained from the analysis of *P. lividus* larvae exposed to the sunscreen SS3 and the control.

		CONTROL				SS3			
Element	An	C un (wt. %)	C norm (wt. %)	C atom (at. %)	Error (wt. %)	C un (wt. %)	C norm (wt. %)	C atom (at. %)	Error (wt. %)
Ca	20	3.37	32.67	30.57	0.17	3.67	29.17	27.06	0.16
Cl	17	0.64	6.24	6.60	0.07	1.26	10.00	10.48	0.08
Cu	29	2.12	20.54	12.12	0.22	1.76	14.02	8.20	0.14
Mg	12	0.48	4.70	7.25	0.08	0.76	5.81	9.15	0.09
P	15	1.61	15.65	18.95	0.12	1.79	13.72	16.94	0.11
S	16	1.53	14.90	17.42	0.11	1.79	13.71	16.35	0.11
Si	14	0.55	5.30	7.08	0.07	0.54	4.30	5.69	0.05
Ti	22	n.d	n.d	n.d	n.d	0.56	4.28	3.42	0.07
Zn	30	n.d	n.d	n.d	n.d	0.65	5.00	2.92	0.10

EDS were sampled in proximity to the gut of each larva. Lead, beam energy 20 keV, identifying the corresponding chemical elements: C, Cu Ca, P, Ti, Mg, Cl, Si, S, P, and Zn. An = Atomic Number; C un [wt.%] = the unnormalized concentration in weight percent of the element; C no [wt.%] = the normalised concentration in weight percent of the element; C Atom. [at. %] = the atomic weight percent; n.d. not detected.

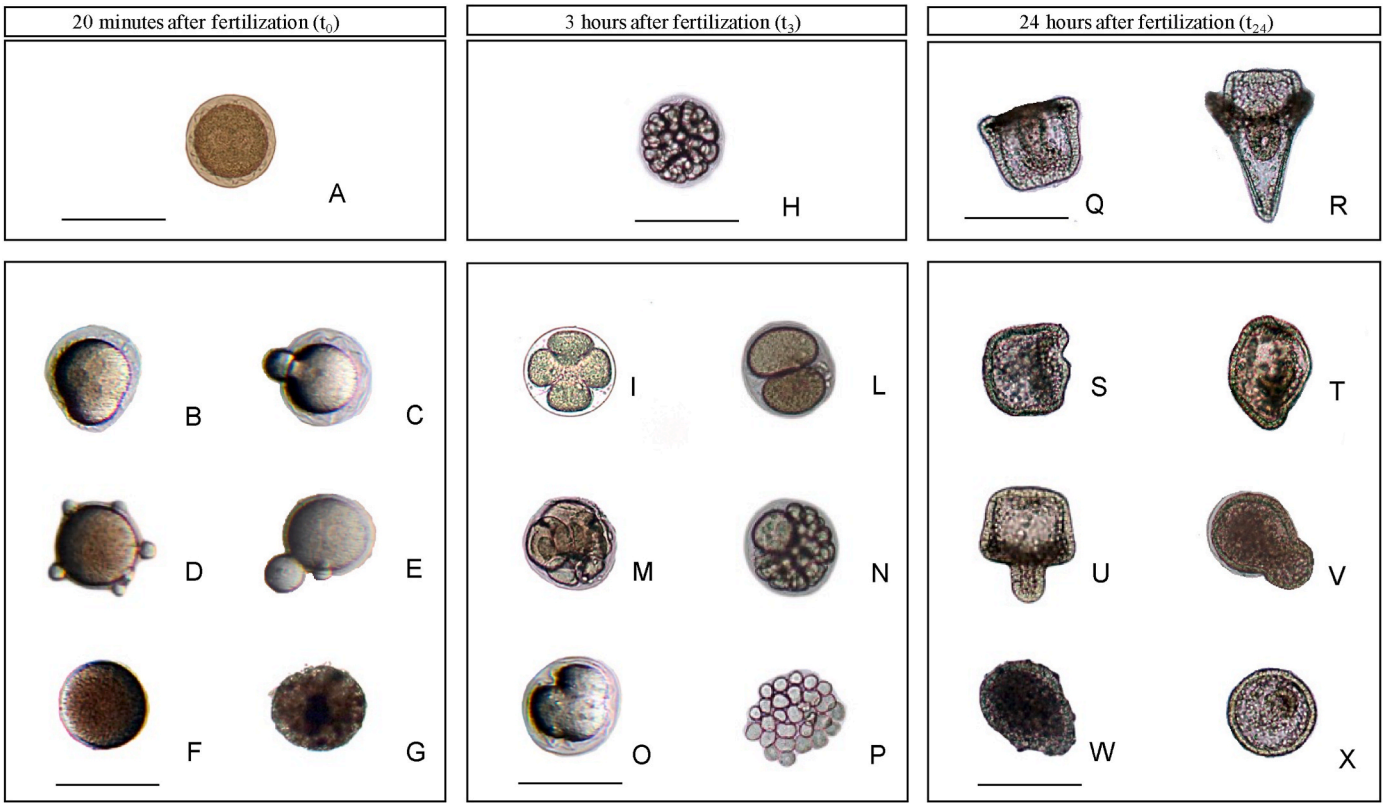


Fig. 4. *P. lividus* developmental anomalies due to early exposure to four sunscreens and Zinc oxide (low and high concentrations) at 20 min, 3 and 24 h post fertilization. Unexposed embryos: normal zygote showing fertilisation membrane and hyaline layer at the start of the experiment (A) 20 min after fertilization; normal embryos at morula stage (H) 3 h after fertilization; normal embryos at prism and pre-pluteus stage (Q–R) 24 h after fertilization. Anomalous aspects of damaged eggs 20 min after fertilization with destabilization of the fertilization membrane (B–C), blebs on the surface (D–E), unfertilized egg (F), signs of cell necrosis (G). Undeveloped embryo at the morula stage (3 h): blocked at two and four cells' stages (I–L), asymmetrical division into blastomeres (M–O), distruption of cell adhesion in morula stage, followed by embryonic disaggregation (P). Anomalous embryos at 24 h of exposure: gastrulae with irregular shape due to anomalous migration of the primary mesenchyme (S–T), exogastrula (U–V), embryo in an advanced stage of necrosis (W), severe delay, embryo at early gastrula stage (X). Scale bar: 100 μm.

developmental delay at the early gastrula stage (Fig. 4 X). The same treatments on *P. lividus* larvae caused 68.0% and 80.7 % of anomalous larvae, respectively (Table S5). Consequently, the high concentration of nano ZnO resulted in a high ICI* value (1.26) since 60.2% of anomalous larvae were classified as level 2, and 12.9% as level 3 (Table S5). Low concentration of nano ZnO caused an ICI* value of 0.92 (Table S5), with a higher frequency of larval anomalies of levels 2 (53.1 %) and 3 (5.8 %). The main developmental abnormalities observed after 24 h of exposure at low and high nano ZnO concentrations were: severe developmental delay (Fig. 5 B), skeletal anomalies in particular shorter spicules leading to a reduction of arm's length, asymmetric skeletal pattern (Fig. 5 C, D, L and M), fractured ectoderm, absence of skeletal rods and folded tips (Fig. 5 G), irregular, joined or reduced arms (Fig. 5 E, F, H), irregular shape and swollen gut and/or anus (Fig. 5 H, I) and evidence of necrosis signs (Fig. 5 N).

Twenty-four hours after the start of the experiment, numerous red amoebocytes were observed within the larvae exposed to the sunscreens containing inorganic UV filters, especially at high concentrations of nano ZnO (Fig. 3).

4. Discussion

4.1. Effects of inorganic UV filter-based sunscreens on early developmental stages of P. lividus

Although the negative effects of inorganic UV filters have been documented for different marine organisms (Li et al., 2018; Cunningham et al., 2020; Mazur et al., 2020; Prato et al., 2021), sunscreen products containing such filters are still present in the market and proposed as safer substitutes of harmful sunscreens based on organic UV filters (Schneider and Lim, 2019; Babarus et al., 2023), as in the case of those containing oxybenzone and octinoxate, which have been banned in different countries around the world (Barone et al., 2019; Raffa et al., 2019).

Here we tested the effects of inorganic UV filter-based sunscreen products labelled as eco-friendly (SS1, SS3 and SS4) on the embryonic and larval development of *Paracentrotus lividus*.

In particular, the experiments were conducted on embryos and larvae because previous studies revealed high sensitivity of embryos and larvae to contaminants, including sunscreens, but the responses to toxicity changed depending on when the offspring came into contact with these contaminants (Burić et al., 2015; Corinaldesi et al., 2017; Gambardella et al., 2021).

These effects were compared with those caused by an eco-certified sunscreen product (SS2) based on new-generation organic UV filters (e.g. ethylhexyl triazone, methylene bis-benzotriazolyl tetra-methyl-butylphenol, proposed as eco-friendly alternatives (Thorel et al., 2020; Varrella et al., 2022).

Previous studies reported that products containing these UV filters did not affect the early developmental stages of sea urchins (Corinaldesi et al., 2017).

We found that the severity of the effects of all treatments on the embryos and larvae of *P. lividus* depended on the product/ingredient tested. However, similar malformations were observed after the

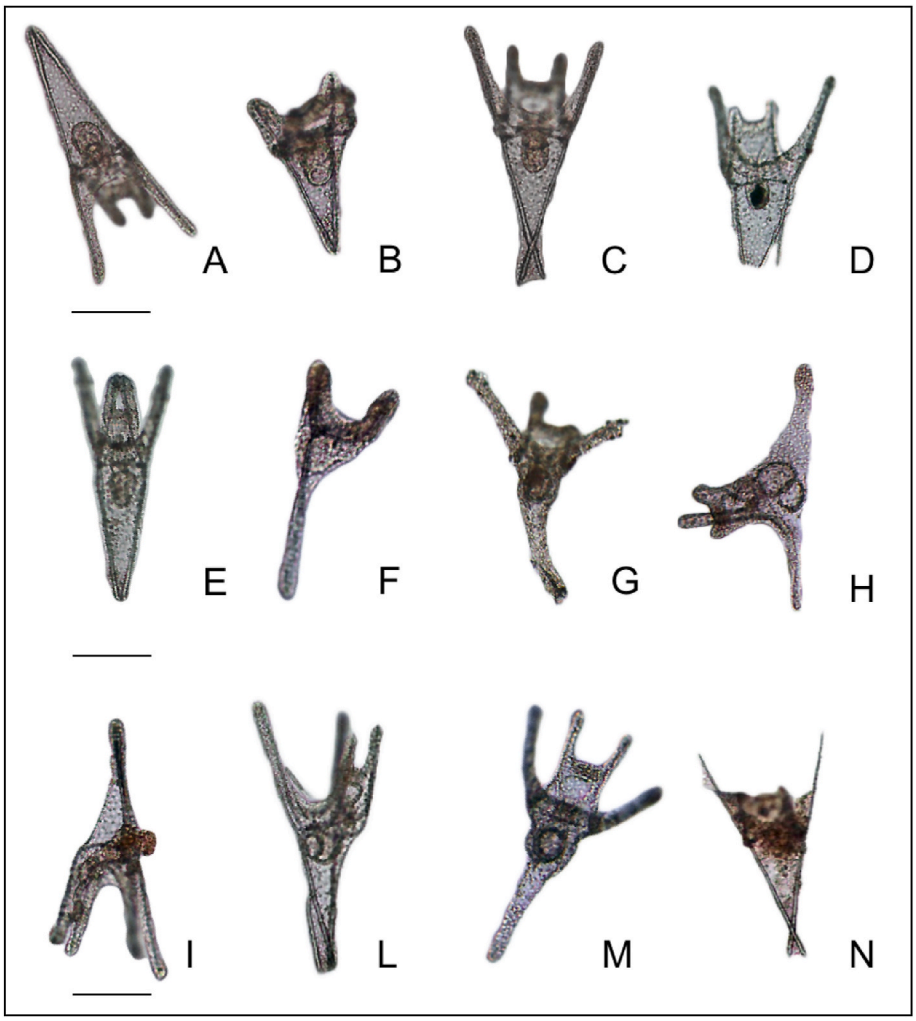


Fig. 5. *P. lividus* larval developmental anomalies due to late exposure (pluteus stage) to four sunscreens and Zinc oxide (low and high concentrations) for 24 h. Healthy pluteus unexposed (A); developmental delay, early pluteus (B); larva with crossed skeletal tips at the hood apex (C), larva characterized to separated skeletal tips at the hood apex (D); larva with joined anterior arms (E); larva with reduced or missing arms (F); larva with fractured ectoderm, absence of skeletal rods and folded tip (G); larva with folded arms, irregolar shape and swollen intestine and anus (H); larva with irregular gut (I); larva with ramified rods (L); larva with incomplete skeletal rods and thin body (M); dead larvae with evidence sign of necrosis and crossed tips (N). Scale bar: 100 μm.

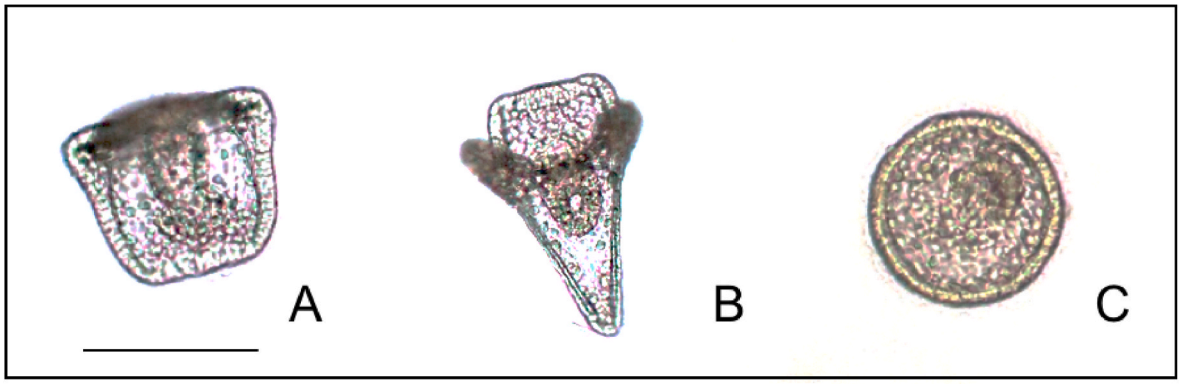


Fig. 6. *P lividus* anomalous embryos and control exposed to sunscreen products SS3 and SS4, 24 h after fertilization. Untreated embryos (A, B) and embryos with severe developmental delay, at early gastrula (C). Scale bars 100 μm.

treatment with all products containing inorganic UV filters.

Among the tested sunscreen products, the sunscreen SS4, containing nano TiO_2 and organic UV filters (i.e. octocrylene, homosalate, ethylhexyl salicylate, butyl methoxydibenzoylmethane) and the sunscreen SS3, characterized by TiO_2 and ZnO mixture (non-nano), caused significant and adverse impacts on the early development of the sea urchin.

In terms of the abundance of abnormal embryos, the negative effect of these products was immediate and evident already since the

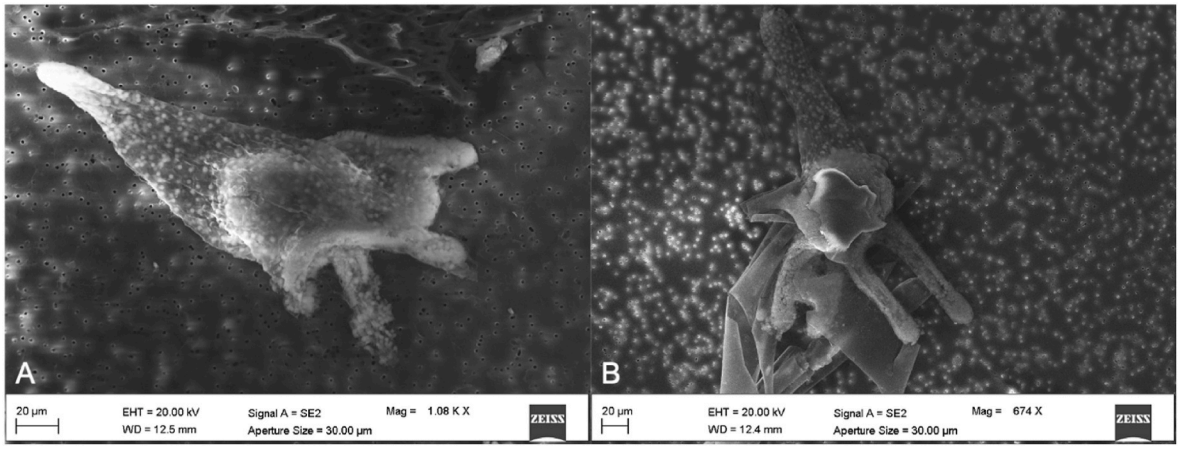


Fig. 7. Morphological details of sea urchin larvae obtained with Scanning electron microscopy (SEM). *P. lividus* larva grown in filtered seawater, used as a control (A) and after 24 h of exposure to solar product SS3 at a concentration of 50 µl L^{-1} (B). Scale bar 20 µ m.

fertilisation with negative effects that worsened over time. The highest vulnerability of the sea urchin embryos to SS3 and SS4 was also confirmed by ICI* values and the results of the morphological analysis, which revealed the highest percentage of delayed embryos at the early gastrula stage and necrosis. The developmental delay at the gastrula stage appears to be a consequence of an altered energy budget invested in the defence systems against contaminants rather than in development (Gambardella et al., 2013; Bonaventura et al., 2022). Necrosis, which is typically due to early exposure to contaminants, occurs because of an acute toxicity event or in relation to their accumulation (Gambardella et al., 2021; Chiarelli et al., 2022).

We argue that the *P. lividus* embryos exposed to sunscreens containing nano TiO_2 and organic UV filters caused a reduction of their growth rate (Roepke et al., 2005) thus affecting the fitness of the population (Gambardella et al., 2021).

While the sunscreen product based on a mixture of non-nano ZnO and TiO_2 (SS3) and the one containing organic UV filters and nano TiO_2 (SS4) determined a similar impact on the embryos, the larval development was more sensitive to SS4 than SS3.

The organic UV filters contained in SS4 have been already demonstrated to be harmful to marine organisms (Bachelot et al., 2012; Lozano et al., 2020; Thorel et al., 2020).

At the same time, previous investigations reported that TiO_2 can be toxic to marine organisms depending on its crystal form (e.g. rutile vs anatase) and particle size (e.g. nanoparticles vs non-nanoparticles) (Sánchez-Quiles and Tovar-Sánchez, 2014; Catalano et al., 2020; Saini and Kumar, 2023). Furthermore, the toxicity of nano TiO_2 can be due to the combination of TiO_2 nanoparticles themselves and the –OH radicals generated, which can cause oxidative damage to cell membranes (Xiong et al., 2011), genotoxic damage (Sendra et al., 2017), and developmental defects in marine invertebrates (Ignoto et al., 2023; Palmeira-Pinto et al., 2023).

SS4 also contains silicones, such as cyclopentasiloxane, which have been reported to be harmful to marine organisms (Bachelot et al., 2012; Gago-Ferrero et al., 2013).

We argue that the combination of nano TiO_2 with organic UV filters, and possibly also their interaction with other ingredients, cause major impacts on the early development and therefore expected survival of sea urchins.

Based on the results presented here the sunscreen SS4 cannot be defined as eco-compatible despite its “safe sea” claim. Likewise, the sunscreen SS3 caused a severe impact on sea urchin embryos, not reflecting its eco-compatibility claim (i.e. biodegradable and reef safe) although we did not assess the effects on reef organisms.

These findings highlight the importance of discriminating the concept of biodegradability from that of eco-compatibility. Biodegradability generally is established by cosmetic companies using 10 or 28-day-assays (Regulation (CE) n. 66/2010). Our results indicate that almost 100% of embryos were impaired by 24 h, even according to other scientific investigations (Morroni et al., 2019; Burić et al., 2023).

The observed impact of SS3 could be due to the mixture of different inorganic UV filters (non-nano TiO_2 and ZnO) (de la Vega et al., 2019), which might determine an increase in the reactive oxygen species levels (Sánchez-Quiles and Tovar-Sánchez, 2014) compared to those generated by a single inorganic UV filter. In addition, embryo malformations observed following SS3 exposure, have been linked to the concentration of Zn ions internalized (Cunningham et al., 2020; Aruoja et al., 2009) due to the rapid solubility of Zn^{2+} ions in seawater (Xiong et al., 2011).

Although several studies have focused on the toxicity mechanisms of nanoparticles (Lozano et al., 2020), our results in line with other research (Oliviero et al., 2017) suggest that also non-nano inorganic UV filters can affect marine species.

In contrast to SS3 and SS4, SS1 caused just a slight impact on sea urchin larvae and a null impact on the embryos, resulting in an eco-compatible product. This sunscreen product is characterized by declared eco-friendly ingredients and contains non-nano TiO_2 with alumina, which has the scope to reduce the potential reactivity of photoactivated TiO_2 particles (Gackowski et al., 2023). Likely, the milder impact of TiO_2 in SS1 is also associated with these factors. However, our findings suggest that from the particle size of the UV filters (nano vs. non-nano) is not possible to rule out the impact degree, especially when they are combined with other UV filters and ingredients.

The sunscreen SS2 without inorganic UV filters but containing new-generation organic UV filters (ethylhexyl triazone and methylene bis-benzotriazolyl tetramethylbutylphenol) did not cause any negative impact on sea urchins, confirming its fully eco-compatibility (Varrella et al., 2022).

Overall, the results of this study reveal that among the sunscreen products tested here, labelled as eco-compatible, those containing ZnO and TiO_2 cannot be considered a more eco-friendly alternative to sunscreens based on harmful organic UV filters without an accurate assessment of their impacts on marine organisms such as *P. lividus*.

4.2. *Exploring the mechanisms of the impact of inorganic UV filters and nano ZnO on early developmental stages of P. lividus*

To better elucidate the effects of inorganic UV filters contained in sunscreen products, we tested the impact of ZnO nanoparticles on sea urchin development.

In the early development stages of sea urchins, the toxicity of ZnO due to dissolved Zn ions and their negative effects on skeletal

calcification has been reported (Manzo et al., 2013; Cunningham et al., 2020; Labille et al., 2020; Mazur et al., 2020; Prato et al., 2021). The most sensitive development stages to Zn ions in the sea urchin appear to be embryonic ones, between fertilization and the mesenchymal blastula when exogastrulation can occur in case of exposure to these ions, which compete with absorption mechanisms of Ca and Mg ions (Martino et al., 2019). Some studies reported that the adverse effects of ZnO nanoparticles could be also associated with the nano-sized material (Manzo et al., 2013; Oliviero et al., 2019), which can be up-taken by embryonic cells thus compromising the gamete quality, influencing molecular mechanisms, and acting as chemosensitizers (Wu et al., 2015; Geneviève et al., 2020). Since a relevant portion of ZnO nanoparticles dissolves gradually in seawater in the first 24 h (at concentrations ranging from 0.1 to 10 mg L^{-1}, Fairbairn et al., 2011) we expect that in our experimental conditions, the negative impacts observed on embryos and larvae are due to both Zn^{2+}ions and the nano-sized material.

In our study, we also observed an increasing negative impact of this inorganic UV filter over time, in terms of the number of anomalous embryos and larvae regardless of tested concentration (high or low). Embryonic anomalies observed during the experiments were represented by block of development at the gastrula, blastula or early stages, severe delay of development, asymmetrical division into blastomeres, and necrosis. A larger fraction of embryonic anomalies due to abnormal migration of primary mesenchymal cells, which formed exogastrulae (Gambardella et al., 2021), was especially found in the embryos exposed to a high concentration of ZnO. Consistently with other results, the anomalies observed in our samples were also detected in other species of sea urchins (*Strongylocentrotus purpuratus* and *Anthocidaris crassispina*) exposed to ZnO and other metals (Cunningham et al., 2020; Kobayashi and Okamura, 2005). Despite the huge impact of ZnO on sea urchin embryos, we also observed a great vulnerability of the larvae, even at low concentrations, as confirmed by the ICI* values.

The larval malformations produced by ZnO were associated with the alteration of normal skeletal development due to the incorrect migration of primary mesenchymal cells (Gambardella et al., 2013; Bonaventura et al., 2022; Cunningham et al., 2020). Since skeletal formation is crucial for the sea urchin morphogenesis and is essential for larval wellbeing and survival, our findings reveal that ZnO, even at the lowest concentrations used in marketed sunscreen products, compromises the possibility of the larvae to mature and contribute to the adult population.

Additional data obtained from SEM/EDX revealed that Zn was incorporated by the *P. lividus* larvae (e.g. after exposure to SS3 for 24 h). This phenomenon was not observed in controls indicating that exposure to Zn may cause the bioaccumulation of this metal within the exposed organisms. The same was observed for Ti, which was detected within larvae exposed to SS3, indicating that also this metal may bioaccumulate within *P. lividus* larvae. A similar process was previously documented in sea urchin embryos (Geneviève et al., 2020), and other marine organisms (Della Torre et al., 2014; Alijagic and Pinsino, 2017; Magesky et al., 2017; Marques-Santos et al., 2018).

Our findings extend current literature information to this keystone species and suggest that sunscreen products containing inorganic UV filters may cause the bioaccumulation of metals within larvae, potentially increasing their adverse effects even in organisms that survived to the end of larval development.

Additional microscopic observations revealed that larvae exposed for 24 h (4-arm plutei) to sunscreens containing inorganic UV filters presented numerous red amebocytes (i.e. cells contained in coelomocytes, playing a crucial role in the immune system of echinoderms; Smith et al., 2006; Pinsino et al., 2007), suggesting the activation of a defence mechanism against inorganic UV filters. In particular, red amoebocytes increased in number when the larvae were exposed to high concentrations of ZnO nanoparticles, which caused the greatest detrimental effects even compared to the complete sunscreen product tested. The biological effect observed here reflects the sea urchin response in systems subject to multiple stressful conditions (Matranga et al., 2005, 2000; Falugi et al., 2012; Pinsino and Matranga, 2015; Stabili and Pagliara, 2015; Magesky et al., 2017; Manzo et al., 2017).

Taken together, our results suggest that inorganic UV filters contained in commercial sunscreens can determine the rapid accumulation of Zn and Ti within the larvae of marine species and induce anomalies in skeletal development and tissue necrosis (Roma et al., 2020; Palmeira-Pinto et al., 2023).

Since in the sea urchin larvae exposed to ZnO and sunscreens with inorganic UV filters (SS1, SS3 and SS4) the observed larval anomalies were similar, we hypothesise that inorganic UV filters, especially ZnO (both nano and non-nano), are responsible for such malformations.

However, we cannot exclude a possible synergistic effect of other ingredients present in sunscreen formulations that can impair the normal development of sea urchins (Corinaldesi et al., 2017).

5. Conclusions

Today's cosmetic industry is moving towards the use of inorganic UV filters in sunscreen products as safer alternatives to conventional organic UV filter-based products due to human health and environmental concerns. Our findings show that the eco-compatibility claim of sunscreen products containing inorganic UV filters can be unsupported in sunscreen products containing nanoparticles of Zn and Ti oxides for their impact on marine organisms. The results reported here prove, indeed, that ZnO and TiO_2-containing sunscreen products hamper the development and fitness of sea urchin populations. This investigation also corroborates the need to improve the eco-friendliness assessment of sunscreen products and warns of the risk of bioaccumulation and potential biomagnification of inorganic UV filters along the marine food chain.

CRediT authorship contribution statement

F. Marcellini: Writing – review & editing, Writing – original draft, Visualization, Data curation. **S. Varrella:** Writing – review & editing, Writing – original draft, Visualization. **M. Ghilardi:** Writing – review & editing, Investigation, Data curation. **G. Barucca:** Writing – review & editing, Data curation. **A. Giorgetti:** Writing – review & editing, Data curation. **R. Danovaro:** Writing – review & editing, Resources, Funding acquisition. **C. Corinaldesi:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests Cinzia Corinaldesi reports financial support was provided by Polytechnic University of Marche. Roberto Danovaro reports financial support was provided by Polytechnic University of Marche. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank Prof. Carla Falugi for the precious suggestions during the experiments.

This work was supported by the National Recovery and Resilience Plan (NRRP) of the Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Project code CN_00000033 - National Biodiversity Future Center - NBFC. LAL acknowledges support from NSF OCE-2048720. The work was also produced in the framework

of the Horizon Europe Programme Project DiverSea (grant agreement N. 101082004) supported by the European Community.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124093.

References

Adler, B.L., DeLeo, V.A., 2020. Sunscreen safety: a review of recent studies on humans and the environment. Current Dermatology Reports 9, 1–9. https://doi.org/10.1007/s13671-020-00284-4.

Alijagic, A., Pinsino, A., 2017. Probing safety of nanoparticles by outlining sea urchin sensing and signaling cascades. Ecotoxicol. Environ. Saf. 144, 416–421. https://doi.org/10.1016/j.ecoenv.2017.06.060.

Amberg, N., Fogarassy, C., 2019. Green consumer behavior in the cosmetics market. Resources 8 (3), 137. https://doi.org/10.3390/resources8030137.

Amemiya, S., 1996. Complete regulation of development throughout metamorphosis of sea urchin embryos devoid of macromeres. Dev. Growth Differ. 31, 131–145. https://doi.org/10.1046/j.1440-169X.1996.t01-4-00003.x.

Anderson, M.J., 2005. Permutational multivariate analysis of variance. Department of Statistics. University, of Auckland, Auckland.

Aruoja, V., Dubourguier, H.C., Kasemets, K., Kahru, A., 2009. Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae Pseudokirchneriella subcapitata. Sci. Total Environ. 407 (4), 1461–1468. https://doi.org/10.1016/j.scitotenv.2008.10.053.

Babarus, I., Lungu, I.I., Stefanache, A., 2023. The dynamic duo: titanium dioxide and zinc oxide in uv-protective cosmetic. https://doi.org/10.37118/ijdr.27134.09.2023.

Bachelot, M., Li, Z., Munaron, D., Le Gall, P., Casellas, C., Fenet, H., Gomez, E., 2012. Organic UV filter concentrations in marine mussels from French coastal regions. Sci. Total Environ. 420, 273–279. https://doi.org/10.1016/j.scitotenv.2011.12.051.

Barone, A.N., Hayes, C.E., Kerr, J.J., Lee, R.C., Flaherty, D.B., 2019. Acute toxicity testing of TiO_2-based vs. oxybenzone-based sunscreens on clownfish (*Amphiprion ocellaris*). Environ. Sci. Pollut. Control Ser. 26, 14513–14520. https://doi.org/10.1007/s11356-019-04769-z.

Blasco, J., Trombini, C., Sendra, M., Araujo, C.V., 2020. Environmental risk assessment of sunscreens. Sunscreens in Coastal Ecosystems: Occurrence, Behavior, Effect and Risk 163–184.

Board, O.S., National Academies of Sciences, Engineering, and Medicine, 2022. Review of fate, exposure, and effects of sunscreens in aquatic environments and implications for sunscreen usage and human health. https://doi.org/10.17226/26381.

Bonaventura, R., Costa, C., Deidda, I., Zito, F., Russo, R., 2022. Gene expression analysis of the stress response to lithium, nickel, and zinc in *Paracentrotus lividus* embryos. Toxics 10 (6), 325. https://doi.org/10.3390/toxics10060325.

Boudouresque, C.F., Verlaque, M., 2020. Paracentrotus lividus. Dev. Aquacult. Fish. Sci. 43, 447–485. https://doi.org/10.1016/B978-0-12-819570-3.00026-3. Elsevier.

Burić, P., Jakšić, Ž., Štajner, L., Sikirić, M.D., Jurašin, D., Cascio, C., et al., 2015. Effect of silver nanoparticles on Mediterranean sea urchin embryonal development is species specific and depends on moment of first exposure. Mar. Environ. Res. 111, 50–59. https://doi.org/10.1016/j.marenvres.2015.06.015.

Burić, P., Čarapar, I., Pavičić-Hamer, D., Kovačić, I., Jurković, L., Dutour Sikirić, M., et al., 2023. Particle size modulates silver nanoparticle toxicity during embryogenesis of urchins *Arbacia lixula* and *Paracentrotus Lividus*. Int. J. Mol. Sci. 24 (1), 745. https://doi.org/10.3390/ijms24010745.

Carballeira, C., Ramos-Gómez, J., Martín-Díaz, L., DelValls, T., 2012. Identification of specific malformations of sea urchin larvae for toxicity assessment: application to marine pisciculture effluents. Mar. Environ. Res. 77, 12–22. https://doi.org/10.1016/j.marenvres.2012.01.001.

Catalano, R., Labille, J., Gaglio, D., Alijagic, A., Napodano, E., Slomberg, D., et al., 2020. Safety evaluation of TiO_2 nanoparticle-based sunscreen UV filters on the development and the immunological state of the sea urchin *Paracentrotus Lividus*. Nanomaterials 10 (11), 2102. https://doi.org/10.3390/nano10112102.

Chiarelli, R., Scudiero, R., Memoli, V., Roccheri, M.C., Martino, C., 2022. Toxicity of vanadium during development of sea urchin embryos: bioaccumulation, calcium depletion, ERK modulation and cell-selective apoptosis. Int. J. Mol. Sci. 23 (11), 6239. https://doi.org/10.3390/ijms23116239.

Clarke, K.R., Gorley, R.N., 2006. PRIMER V6: User Manual/Tutorial. PRIMER-E, Plymouth.

Corinaldesi, C., Damiani, E., Marcellini, F., Falugi, C., Tiano, L., Brugè, F., Danovaro, R., 2017. Sunscreen products impair the early developmental stages of the sea urchin *Paracentrotus lividus*. Sci. Rep. 7, 7815. https://doi.org/10.1038/s41598-017-08013-x.

Corinaldesi, C., Marcellini, F., Nepote, E., Damiani, E., Danovaro, R., 2018. Impact of inorganic UV filters contained in sunscreen products on tropical stony corals (*Acropora spp.*). Sci. Total Environ. 637–638, 1279–1285. https://doi.org/10.1016/j.scitotenv.2018.05.108.

Cosmetic Europe annual report, 2020. Available on line: https://cosmeticseurope.eu/files/5716/2373/9480/Cosmetics_Europe_Activity_Report_2020.pdf. (Accessed 9 January 2024).

Cunningham, B., Torres-Duarte, C., Cherr, G., Adams, N., 2020. Effects of three zinc-containing sunscreens on development of purple sea urchin (*Strongylocentrotus purpuratus*) embryos. Aquat. Toxicol. 218, 105355 https://doi.org/10.1016/j.aquatox.2019.105355.

Danovaro, R., Bongiorni, L., Corinaldesi, C., Giovannelli, D., Damiani, E., Astolfi, P., Greci, L., Pusceddu, A., 2008. Sunscreens cause coral bleaching by promoting viral infections. Environ. Health Perspect. 116, 441–447. https://doi.org/10.1289/ehp.10966.

de la Vega, A.C.S., Molins-Delgado, D., Barceló, D., Díaz-Cruz, M.S., 2019. Nanosized titanium dioxide UV filter increases mixture toxicity when combined with parabens. Ecotoxicol. Environ. Saf. 184, 109565 https://doi.org/10.1016/j.ecoenv.2019.109565.

Della Torre, C., Bergami, E., Salvati, A., Faleri, C., Cirino, P., Dawson, K.A., Corsi, I., 2014. Accumulation and embryotoxicity of polystyrene nanoparticles at early stage of development of sea urchin embryos Paracentrotus lividus. Environ. Sci. Technol. 48 (20), 12302–12311. https://doi.org/10.1021/es502569w.

Ecolabel Report, 2021. Available on line:. EU Commission https://environment.ec.europa.eu/topics/circular-economy/eu-ecolabel-home/business/ecolabel-facts-and-figures_en. (Accessed 22 July 2023).

Fairbairn, E.A., Keller, A.A., Mädler, L., Zhou, D., Pokhrel, S., Cherr, G.N., 2011. Metal oxide nanomaterials in seawater: linking physicochemical characteristics with biological response in sea urchin development. J. Hazard Mater. 192 (3), 1565–1571. https://doi.org/10.1016/j.jhazmat.2011.06.080.

Falugi, C., Lammerding-Koppel, M., Aluigi, M.G., 2008. Sea urchin development: an alternative model for mechanistic understanding of neurodevelopment and neurotoxicity. Birth Defects Res. Part C Embryo Today - Rev. 84 (3), 188–203. https://doi.org/10.1002/bdrc.20132.

Falugi, C., Aluigi, M.G., Chiantore, M.C., Privitera, D., Ramoino, P., Gatti, M.A., et al., 2012. Toxicity of metal oxide nanoparticles in immune cells of the sea urchin. Mar. Environ. Res. 76, 114–121. https://doi.org/10.1016/j.marenvres.2011.10.003.

Fastelli, P., Renzi, M., 2019. Exposure of key marine species to sunscreens: changing ecotoxicity as a possible indirect effect of global warming. Mar. Pollut. Bull. 149, 110517 https://doi.org/10.1016/j.marpolbul.2019.110517.

Gackowski, M., Osmałek, T., Froelich, A., Otto, F., Schneider, R., Lulek, J., 2023. Phototoxic or photoprotective?—advances and limitations of titanium (IV) oxide in dermal formulations—a review. Int. J. Mol. Sci. 24 (9), 8159. https://doi.org/10.3390/ijms24098159.

Gago-Ferrero, P., Alonso, M.B., Bertozzi, C.P., Marigo, J., Barbosa, L., Cremer, M., et al., 2013. First determination of UV filters in marine mammals. Octocrylene levels in *Franciscana dolphins*. Environ. Sci. Technol. 47 (11), 5619–5625. https://doi.org/10.1021/es400675y.

Gambardella, C., Aluigi, M.G., Ferrando, S., Gallus, L., Ramoino, P., Gatti, A.M., et al., 2013. Developmental abnormalities and changes in cholinesterase activity in sea urchin embryos and larvae from sperm exposed to engineered nanoparticles. Aquat. Toxicol. 130, 77–85. https://doi.org/10.1016/j.aquatox.2012.12.025.

Gambardella, C., Marcellini, F., Falugi, C., Varrella, S., Corinaldesi, C., 2021. Early-stage anomalies in the sea urchin (*Paracentrotus lividus*) as bioindicators of multiple stressors in the marine environment: overview and future perspectives. Environ. Pollut. 287, 117608 https://doi.org/10.1016/j.envpol.2021.117608.

Geneviève, A.M., Derelle, E., Escande, M.L., Grimsley, N., Klopp, C., Ménager, C., et al., 2020. Responses to iron oxide and zinc oxide nanoparticles in echinoderm embryos and microalgae: uptake, growth, morphology, and transcriptomic analysis. Nanotoxicology 14 (10), 1342–1361. https://doi.org/10.1080/17435390.2020.1827074.

Giudice, G., 1986. The Sea Urchin Embryo: a Developmental Biological System. Springer-Verlag Berlin Heidelberg, Berlin, Heidelberg.

Hojerová, J., Medovcíková, A., Mikula, M., 2011. Photoprotective efficacy and photostability of fifteen sunscreen products having the same label SPF subjected to natural sunlight. Int. J. Pharm. 408 (1–2), 27–38. https://doi.org/10.1016/j.ijpharm.2011.01.040.

Ignoto, S., Pecoraro, R., Scalisi, E.M., Contino, M., Ferruggia, G., Indelicato, S., et al., 2023. Spermiotoxicity of nano-TiO_2 compounds in the sea urchin *Paracentrotus lividus* (lamarck, 1816): considerations on water remediation. J. Mar. Sci. Eng. 11 (2), 380. https://doi.org/10.3390/jmse11020380.

Keller, A.A., 2023. Nanomaterials in sunscreens: potential human and ecological health implications. Int. J. Cosmet. Sci. 45, 127–140. https://doi.org/10.1111/ics.12905.

Khosravi-Katuli, K., Lofrano, G., Nezhad, H.P., Giorgio, A., Guida, M., Aliberti, F., et al., 2018. Effects of ZnO nanoparticles in the Caspian roach (Rutilus rutilus caspicus). Sci. Total Environ. 626, 30–41. https://doi.org/10.1016/j.scitotenv.2018.01.085.

Kobayashi, N., Okamura, H., 2005. Effects of heavy metals on sea urchin embryo development. Part 2. Interactive toxic effects of heavy metals in synthetic mine effluents. Chemosphere 61 (8), 1198–1203. https://doi.org/10.1016/j.chemosphere.2005.02.071.

Labille, J., Slomberg, D., Catalano, R., Robert, S., Apers-Tremelo, M.L., Boudenne, J.L., et al., 2020. Assessing UV filter inputs into beach waters during recreational activity: a field study of three French Mediterranean beaches from consumer survey to water analysis. Sci. Total Environ. 706, 136010 https://doi.org/10.1016/j.scitotenv.2019.136010.

Lead, J.R., Batley, G.E., Alvarez, P.J., Croteau, M.N., Handy, R.D., McLaughlin, M.J., Schirmer, K., 2018. Nanomaterials in the environment: behavior, fate, bioavailability, and effects—an updated review. Environ. Toxicol. Chem. 37 (8), 2029–2063. https://doi.org/10.1002/etc.4147.

Li, J., Chen, Z., Huang, R., Miao, Z., Cai, L., Du, Q., 2018. Toxicity assessment and histopathological analysis of nano-ZnO against marine fish (*Mugilogobius chulae*) embryos. J. Environ. Sci. 73, 78–88. https://doi.org/10.1016/j.jes.2018.01.015.

Libralato, G., Minetto, D., Totaro, S., Mičetić, I., Pigozzo, A., Sabbioni, E., Marcomini, A., Volpi Ghirardini, A., 2013. Embryotoxicity of TiO_2 nanoparticles to *Mytilus galloprovincialis* (lmk). Mar. Environ. Res. 92, 71–78. https://doi.org/10.1016/j.marenvres.2013.08.015.

Lozano, C., Givens, J., Stien, D., Matallana-Surget, S., Lebaron, P., 2020. Bioaccumulation and toxicological effects of UV-filters on marine species. Sunscreens in coastal ecosystems: Occurrence, behavior, effect and risk 85–130. https://doi.org/10.1007/698_2019_442.

Magesky, A., de Oliveira Ribeiro, C.A., Beaulieu, L., Pelletier, É., 2017. Silver nanoparticles and dissolved silver activate contrasting immune responses and stress-induced heat shock protein expression in sea urchin. Environ. Toxicol. Chem. 36 (7), 1872–1886. https://doi.org/10.1002/etc.3709.

Manzo, S., Miglietta, M.L., Rametta, G., Buono, S., Di Francia, G., 2013. Embryotoxicity and spermiotoxicity of nanosized ZnO for Mediterranean sea urchin Paracentrotus lividus. J. Hazard Mater. 254, 1–9. https://doi.org/10.1016/j.jhazmat.2013.03.027.

Manzo, S., Schiavo, S., Oliviero, M., Toscano, A., Ciaravolo, M., Cirino, P., 2017. Immune and reproductive system impairment in adult sea urchin exposed to nanosized ZnO via food. Sci. Total Environ. 599–600, 9–13. https://doi.org/10.1016/j.scitotenv.2017.04.173.

Marques-Santos, L.F., Grassi, G., Bergami, E., Faleri, C., Balbi, T., Salis, A., et al., 2018. Cationic polystyrene nanoparticle and the sea urchin immune system: biocorona formation, cell toxicity, and multixenobiotic resistance phenotype. Nanotoxicology 12 (8), 847–867. https://doi.org/10.1080/17435390.2018.1482378.

Martino, C., Chiarelli, R., Roccheri, M.C., Matranga, V., Byrne, M., 2019. Effects of magnesium deprivation on development and biomineralization in the sea urchin *Arbacia lixula*. Invertebr. Reprod. Dev. 63 (3), 165–176. https://doi.org/10.1080/07924259.2019.1611670.

Matranga, V., Toia, G., Bonaventura, R., Müller, W.E., 2000. Cellular and biochemical responses to environmental and experimentally induced stress in sea urchin coelomocytes. Cell stress & chaperones 5 (2), 113120. https://doi.org/10.1379/14661268(2000)005<0113:CABRTE>2.0.CO;2.

Matranga, V., Pinsino, A., Celi, M., Natoli, A., Bonaventura, R., Schröder, H.C., Müller, W.E.G., 2005. Monitoring chemical and physical stress using sea urchin immune cells. Echinodermata 85–110. https://doi.org/10.1007/3-540-27683-1_5.

Mazur, A.A., Zhuravel, E.V., Slobodskova, V.V., Mazur, M.A., Kukla, S.P., Chelomin, V.P., 2020. Waterborne exposure of adult sand dollar, *scaphechinus mirabilis* (agassiz, 1864), to zinc ions and zinc oxide nanoparticles affects early development of its offspring. Water Air Soil Pollut. 231, 115. https://doi.org/10.1007/s11270-020-04484-3.

McArdle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82, 290–297. https://doi.org/10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO;2.

Mejjad, N., Rossi, A., Pavel, A.B., 2022. The coastal tourism industry in the Mediterranean: a critical review of the socio-economic and environmental pressures & impacts. Tourism Manag. Perspect. 44, 101007 https://doi.org/10.1016/j.tmp.2022.101007.

Miller, I.B., Pawlowski, S., Kellermann, M.Y., Petersen-Thiery, M., Moeller, M., Nietzer, S., Schupp, P.J., 2021. Toxic effects of UV filters from sunscreens on coral reefs revisited: regulatory aspects for “reef safe” products. Environ. Sci. Eur. 33 (1), 1–13. https://doi.org/10.1186/s12302-021-00515-w.

Morroni, L., Sartori, D., Costantini, M., Genovesi, L., Magliocco, T., Ruocco, N., Buttino, I., 2019. First molecular evidence of the toxicogenetic effects of copper on sea urchin Paracentrotus lividus embryo development. Water Res. 160, 415–423. https://doi.org/10.1016/j.watres.2019.05.062.

Naik, M.S., Polad, R.J., Joshi, M.M., Ligade, V.S., 2022. Global trends of sunscreen research literature: a bibliometric analysis over the period of 2010–2020. J. Cutan. Aesthetic Surg. 15 (2), 161. https://doi.org/10.4103/JCAS.JCAS_110_21.

Oliviero, M., Schiavo, S., Rametta, G., Miglietta, M.L., Manzo, S., 2017. Different sizes of ZnO diversely affected the cytogenesis of the sea urchin Paracentrotus lividus. Sci. Total Environ. 607, 176–183. https://doi.org/10.1016/j.scitotenv.2017.07.038.

Oliviero, M., Schiavo, S., Dumontet, S., Manzo, S., 2019. DNA damages and offspring quality in sea urchin Paracentrotus lividus sperms exposed to ZnO nanoparticles. Sci. Total Environ. 651, 756–765. https://doi.org/10.1016/j.scitotenv.2018.09.243.

Palmeira-Pinto, L., Emerenciano, A.K., Bergami, E., Joviano, W.R., Rosa, A.R., Neves, C. L., et al., 2023. Alterations induced by titanium dioxide nanoparticles (nano-TiO_2) in fertilization and embryonic and larval development of the tropical sea urchin Lytechinus variegatus. Mar. Environ. Res. 188, 106016 https://doi.org/10.1016/j.marenvres.2023.106016.

Pawlowski, S., Luetjens, L.H., Preibisch, A., Acker, S., Petersen-Thiery, M., 2023. Cosmetic UV filters in the environment—state of the art in EU regulations, science and possible knowledge gaps. Int. J. Cosmet. Sci. 45, 52–66. https://doi.org/10.1111/ics.12898.

Pinsino, A., Matranga, V., 2015. Sea urchin immune cells as sentinels of environmental stress. Dev. Comp. Immunol. 49 (1), 198–205. https://doi.org/10.1016/j.dci.2014.11.013.

Pinsino, A., Thorndyke, M.C., Matranga, V., 2007. Coelomocytes and post-traumatic response in the common sea star *Asterias rubens*. Cell stress & chaperones 12 (4), 331. https://doi:10.1379/csc-288.1.

Prato, E., Fabbrocini, A., Libralato, G., Migliore, L., Parlapiano, I., D'Adamo, R., et al., 2021. Comparative toxicity of ionic and nanoparticulate zinc in the species *Cymodoce truncata, Gammarus aequicauda* and *Paracentrotus lividus*. Environ. Sci. Pollut. Control Ser. 28 (31), 42891–42900. https://doi.org/10.1007/s11356-021-13712-0.

Raffa, R.B., Pergolizzi Jr, J.V., Taylor Jr, R., Kitzen, J.M., NEMA Research Group, 2019. Sunscreen bans: coral reefs and skin cancer. J. Clin. Pharm. Therapeut. 44 (1), 134–139. https://doi.org/10.1111/jcpt.12778.

Roepke, T.A., Snyder, M.J., Cherr, G.N., 2005. Estradiol and endocrine disrupting compounds adversely affect development of sea urchin embryos at environmentally relevant concentrations. Aquat. Toxicol. 71 (2), 155–173. https://doi.org/10.1016/j.aquatox.2004.11.003.

Roma, J., Matos, A.R., Vinagre, C., Duarte, B., 2020. Engineered metal nanoparticles in the marine environment: a review of the effects on marine fauna. Mar. Environ. Res. 105110 https://doi.org/10.1016/j.marenvres.2020.105110.

Saini, R., Kumar, P., 2023. Green synthesis of TiO_2 nanoparticles using Tinospora cordifolia plant extract & its potential application for photocatalysis and antibacterial activity. Inorg. Chem. Commun. 156, 111221 https://doi.org/10.1016/j.inoche.2023.111221.

Sánchez-Quiles, D., Tovar-Sánchez, A., 2014. Sunscreens as a source of hydrogen peroxide production in coastal waters. Environ. Sci. Technol. 48 (16), 9037–9042. https://doi.org/10.1021/es5020696.

Schiavo, S., Oliviero, M., Miglietta, M., Rametta, G., Manzo, S., 2016. Genotoxic and cytotoxic effects of ZnO nanoparticles for Dunaliella tertiolecta and comparison with SiO2 and TiO_2 effects at population growth inhibition levels. Sci. Total Environ. 550, 619–627. https://doi.org/10.1016/j.scitotenv.2016.01.135.

Schneider, S.L., Lim, H.W., 2019. A review of inorganic UV filters zinc oxide and titanium dioxide. Photodermatol. Photoimmunol. Photomed. 35 (6), 442–446. https://doi.org/10.1111/phpp.12439.

Sendra, M., Sánchez-Quiles, D., Blasco, J., Moreno-Garrido, I., Lubián, L.M.M., Pérez-García, S., Tovar-Sánchez, A., 2017. Effects of TiO_2 nanoparticles and sunscreens on coastal marine microalgae: ultraviolet radiation is key variable for toxicity assessment. Environ. Int. 98, 62–68. https://doi.org/10.1016/j.envint.2016.09.024.

Smijs, T.G., Pavel, S., 2011. Titanium dioxide and zinc oxide nanoparticles in sunscreens: focus on their safety and effectiveness. Nanotechnol. Sci. Appl. 95–112. https://doi.org/10.2147/NSA.S19419.

Smith, L.C., Rast, J.P., Brockton, V., Terwilliger, D.P., Nair, S.V., Buckley, K.M., Majeske, A.J., 2006. The sea urchin immune system. Invertebr. Surviv. J. 3 (1), 25–39.

Stabili, L., Pagliara, P., 2015. The sea urchin *Paracentrotus lividus* immunological response to chemical pollution exposure: the case of lindane. Chemosphere 134, 60–66.

Sunscreen Drug Products for Over-the- Counter Human Use, document number 84 FR 6204. Food and Drug Administration. Available on https://www.govinfo.gov/content/pkg/FR-2019-02-26/pdf/2019-03019.pdf.

Thorel, E., Clergeaud, F., Jaugeon, L., Rodrigues, A.M., Lucas, J., Stien, D., Lebaron, P., 2020. Effect of 10 UV filters on the brine shrimp *Artemia salina* and the marine microalga *Tetraselmis sp*. Toxics 8 (2), 29. https://doi.org/10.3390/toxics8020029.

Tovar-Sánchez, A., Sánchez-Quiles, D., Rodríguez-Romero, A., 2019. Massive coastal tourism influx to the Mediterranean Sea: the environmental risk of sunscreens. Sci. Total Environ. 656, 316–321. https://doi.org/10.1016/j.scitotenv.2018.11.399.

Tovar-Sánchez, A., Rodríguez-Romero, A., Engel, A., Zäncker, B., Fu, F., Marañón, E., et al., 2020. Characterizing the surface microlayer in the Mediterranean Sea: trace metal concentrations and microbial plankton abundance. Biogeosciences 17 (8), 2349–2364. https://doi.org/10.5194/bg-17-2349-2020.

Varrella, S., Danovaro, R., Corinaldesi, C., 2022. Assessing the eco-compatibility of new generation sunscreen products through a combined microscopic-molecular approach. Environ. Pollut. 314, 120212 https://doi.org/10.1016/j.envpol.2022.120212.

Wu, B., Torres-Duarte, C., Cole, B.J., Cherr, G.N., 2015. Copper oxide and zinc oxide nanomaterials act as inhibitors of multidrug resistance transport in sea urchin embryos: their role as chemosensitizers. Environ. Sci. Technol. 49 (9), 5760–5770. https://doi.org/10.1021/acs.est.5b00345.

Xiong, D., Fang, T., Yu, L., Sima, X., Zhu, W., 2011. Effects of nano-scale TiO2, ZnO and their bulk counterparts on zebrafish: acute toxicity, oxidative stress and oxidative damage. Sci. Total Environ. 409 (8), 1444–1452. https://doi.org/10.1016/j.scitotenv.2011.01.015.

Yuan, S., Huang, J., Jiang, X., Huang, Y., Zhu, X., Cai, Z., 2022. Environmental fate and toxicity of sunscreen-derived inorganic ultraviolet filters in aquatic environments: a review. Nanomaterials 12 (4), 699. https://doi.org/10.3390/nano12040699.

Yuan, S., Huang, J., Qian, W., Zhu, X., Wang, S., Jiang, X., 2023. Are physical sunscreens safe for marine life? A study on a coral–zooxanthellae symbiotic system. Environ. Sci. Technol. 57 (42), 15846–15857. https://doi.org/10.1021/acs.est.3c04603.