Comparative effects of chemical and physical sunscreen on fertilization of purple sea urchins (*Strongylocentrotus purpuratus*).

Marilla Lippert[1], Maurice Goodman[2], and Nikki L. Adams[1]

[1]Department of Biological Sciences, California Polytechnic State University, San Luis Obispo, CA 93407

[2]Hopkins Marine Station of Stanford University, Monterey, CA 93950

Corresponding Author: Dr. Nikki L. Adams

Email: nadams@calpoly.edu

Current address:
Department of Biological Sciences and
the Center for Coastal Marine Sciences
California Polytechnic State University
1 Grand Ave.
San Luis Obispo, CA 93407
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ABSTRACT

Organic compound-based “chemical” sunscreens dominate the commercial sunscreen market, but recent research has revealed the ingredients of these products are detrimental to the health of marine organisms. This revelation has led to increased popularity of mineral-based “physical” sunscreens, primarily containing zinc-oxide (ZnO), as environmentally safe alternatives. While they are marketed as environmentally safe, these claims are largely untested, and it is important to consider potential effects of ZnO-based sunscreens on the development of marine organisms. Though Zn is a necessary micronutrient in the ocean, excess Zn is released into marine environments from anthropogenic sources has negative effects on marine life. Many studies have examined effects of various chemical and physical sunscreens separately, but there are no published studies comparing them directly. In this study, we document effects of oxybenzone-based “chemical” sunscreen versus zinc-oxide based “physical” sunscreen on fertilization of the purple sea urchin, *Strongylocentrotus purpuratus*. We demonstrate that exposure of gametes to chemical sunscreen has a significantly more detrimental effect on fertilization success than exposure to a physical sunscreen at low concentrations, and that the physical sunscreen is slightly more detrimental to ova at higher concentrations than the chemical sunscreen. We also observed decreases in fertilization success when both gametes were exposed to either sunscreen, indicating an additive effect. While both sunscreens appear harmful to the development of marine organisms, our results from exposing gametes to the lower, more environmentally relevant levels of sunscreens, suggest that physical sunscreen may be less harmful than chemical sunscreen to sea urchin gametes.

Keywords: Sunscreen, Fertilization, Sea Urchin, Zinc-oxide, Oxybenzone

1. Introduction

Anthropogenic pollution has had negative effects on marine organisms and environments in recent decades (Halpern et al., 2008). Despite near ubiquitous use, personal care products such as sunscreens have only recently become a source of concern as anthropogenic pollutants (Pathak, 1987; Wood, 2018). Sunscreens are typically classified into “physical” sunscreens that use minerals such as zinc (ZnO) and titanium (TiO2) oxide to reflect UV radiation, and “chemical” sunscreens that use organic compounds, most commonly oxybenzone and avobenzone, to absorb UV and radiate it as heat (Wood, 2018). Historically, chemical sunscreens, which absorb into the skin, have been in higher demand than physical sunscreens, which form a thick layer atop the skin. However, recent studies have generated public concern over the impacts of chemical sunscreens on marine life (Wood, 2018). Alternatively, the use of physical sunscreens has increased partly because they are marketed as environmentally safe alternatives and often have labels claiming to be “reef safe”. Few studies to date have evaluated the claim that physical sunscreens are “safer” for marine organisms than chemical sunscreens, and, to the best of our knowledge, none have explicitly compared the effects of zinc-oxide and oxybenzone based sunscreens.

Increasing evidence suggests sunscreen components represent a substantial source of marine pollution in waters of populated beaches (Wood, 2018). Donavaro et al. (2008) estimated that at least 25% of sunscreen applied by beachgoers washes off into the ocean after 20 mins of submersion. Considering this release rate and the increasing rate of coastal tourism, studies estimate between 4,000-14,000 tons of sunscreen could be introduced into reef areas per year (Danovaro et al., 2008; Downs et al., 2015; Wood, 2018). Typically, sunscreen filters (e.g. oxybenzone, zinc-oxide) are found at concentrations of a few parts per trillion (ng/L), however, Downs et al. (2015) reported oxybenzone concentrations of over 1 part per million in the US Virgin Islands. Additionally, due to stricter regulations and improvements to wastewater management, a larger proportion of pollutants into marine environments now come from nonpoint sources such as human bathers and swimmers (Cunningham et al., 2020; Downs et al., 2015). As rates of coastal marine tourism increase, it is becoming increasingly important to characterize the effects that commercial sunscreen products have on marine organisms and environments (Sánchez-Quiles & Tovar-Sánchez, 2015).

Physical sunscreens have become more popular as an increasing number of studies have documented the negative impacts of chemical sunscreens on marine life (EWG, 2018). Danovaro et al. (2008) and Downs et al. (2014; 2015) found many ingredients including forms of benzone cause harm to zooxanthellae and induce bleaching in corals. Similarly, environmentally relevant levels of oxybenzone-containing sunscreens have been shown to slow population growth in various reef biota (McCoshum et al., 2016). Benzophenone, another common ingredient of chemical sunscreens, can cause deformation and mortality in coral planulae (Downs et al., 2015).

Recent studies, however, suggest that physical sunscreens may also be detrimental to the growth and survival of marine life. For example, some physical sunscreens have been shown to cause abnormal embryonic development and increased rates of embryo mortality in zebrafish (Hanigan et al., 2018). In addition, zinc-oxide induces coral bleaching and affects photosynthetic efficiency at 1ppm (Fel et al., 2017). Cunningham et al. (2020) demonstrated zinc from physical sunscreens is internalized by purple sea urchin embryos and inhibits the activity of multi-drug resistance transporters that export other toxins. Another long-term exposure study showed that after 12 weeks exposure to zinc-oxide, mussels experienced increased concentrations of zinc in their tissues as well as increased respiration rates, negatively affecting their growth and survival (Hanna et al., 2013).

Sea urchins are an ecologically important and dominant herbivore in kelp forest ecosystems and are recognized as a model organism for broadcast spawning invertebrates (Adams et al., 2019; Campanale et al., 2011; Pearse, 2006). Sea urchins are easily accessible, and their gametes are relatively easy to obtain (Adams et al., 2019). Sea urchin development has also been closely studied and the stages of sea urchin development are well understood (Giudice, 1973; Giudice, 1986; Gustafson, T. & Wolpert, 1963), making them an ideal species for evaluating the effects of pollutants on fertilization and development. Sea urchin embryos have been used in many studies to observe the effects of zinc and other heavy metals on survival and development (Fairbairn et al., 2011; Kobayashi & Okamura. 2004; Miglietta et al., 2011; Timourian, 1968; Wu et al., 2015).

In this study, we compare effects of chemical (oxybenzone) and physical (zinc-oxide) sunscreens on the fertilization success of the purple sea urchin, *Strongylocentrotus purpuratus*. Despite the popularity of zinc-oxide and oxybenzone as UV filters, to our knowledge, this study is the first to compare the effects of zinc-oxide and oxybenzone-based sunscreens on the fertilization of a marine invertebrate. Additionally, few studies have tested the effects of sunscreen filters on important temperate kelp forest organisms. Like corals, temperate kelp is an indispensable foundation species that provide refuge for a plethora of marine organisms. Kelp forests are one of the world's most productive ecosystems and provide services worth billions of dollars annually (Beaumont et al., 2008; Mann, 1973; Steneck et al., 2002). By evaluating the effects of sunscreens on an important kelp forest species, we aim to address these gaps and improve our understanding of anthropogenic impacts on coastal ecosystems.

2. Methods

2.1 Collection of Adult Sea Urchins

Adult sea urchins, *Strongylocentrotus purpuratus,* were collected from the coast of Goleta, California via SCUBA. Sea urchins were transported to the Cal Poly Pier in Avila, CA, and maintained in flow-through seawater tanks while fed a constant diet of giant kelp (*Macrocystis pyrifera)* until used in experiments.

2.2 Preparation of Sunscreen Solutions

Neutrogena Ultra Sheer dry-touch (SPF 45, 6% oxybenzone) and All Good Sport (SPF 33, 11% zinc-oxide) were used as the chemical and physical sunscreens respectively. Both sunscreens were made into solutions via the following method: 10g of sunscreen was added to 50mL of 0.22 µm filtered seawater (FSW) and heated (~37°C) and stirred at medium speed simultaneously. After 20 mins, the mixture was removed from heat and stirred for another 20 mins. Once mixed, each solution was filtered using Whatman Filter Paper (25µm) to remove large chunks of undissolved sunscreen and to create a starting stock sunscreen solution. While the absolute concentrations of zinc oxide and oxybenzone were not determined for this study, these methods replicate the methods of Cunningham et al. (2020) for sunscreen stock solution preparation, which resulted in a concentration of ~3.77 ppm Zn.

2.3 Spawning and gamete preparation

Spawning of adult sea urchins was induced using 0.55 M KCl (as per Adams et al., 2019). Females were allowed to spawn eggs into FSW at 15°C for 30 mins. Eggs were then washed three times before being aliquoted into experimental beakers. A 5% (v/v FSW) suspension of eggs was prepared and subsequently split into experimental aliquots for all experiments. Sperm was collected dry and stored in a microcentrifuge tube on ice until used in experiments. Both eggs and sperm were evaluated for quality and quantity prior to fertilization.

2.4 Exposure & Sperm Dilution Assay

A sperm dilution assay was performed to determine the best concentration of sperm to achieve robust fertilization of eggs in control samples and to also observe an effect of at least one type of sunscreen in experimental treatments at multiple sperm dilutions. Successful fertilization for each batch of eggs was verified by the presence of the fertilization envelope on at least 90% of the embryos for control eggs. We focused this assay on the physical sunscreen.

Egg solutions (5% in FSW, 1:20 dilution) were split into two treatments, control (no sunscreen) or exposed to a 2% dilution of All Good Sport (physical) sunscreen for 30 mins at 15°C. A sperm solution (0.4% sperm in FSW, 1:250 dilution) was prepared and split into two treatments, control (no sunscreen) or exposed to a 0.8% solution of sunscreen in FSW for 30 mins. Four treatment groups were created: control (no sunscreen exposure of gametes), exposed eggs (eggs exposed, not sperm), sperm exposed (sperm exposed not eggs), both exposed (eggs and sperm exposed). Sperm was added to aliquots of eggs for each treatment in the following final sperm dilutions (sperm to FSW): no sperm, 1:500,000, 1:200,000, 1:100,000, 1:50,000, 1:10,000. After 5 mins, 1% formalin was added to stop the fertilization reactions. Two hundred embryos were scored for fertilization membranes. This experiment was repeated four times.

2.5. Sunscreen Exposure Fertilization Assay

Based on the results of the sperm dilution assay, we selected a sperm dilution of 1:50,000 in FSW for all subsequent experiments to allow for high rates of fertilization in the control as well as expression of sunscreen effects (Figure S1). Additionally, we reduced the sunscreen concentrations used in this study based on the sperm dilution assay, because little to no fertilization was observed in treatments for which both eggs and sperm were exposed. Using a factorial experimental design, we compared the effects of sunscreen types (chemical and physical) at two different concentrations (described below) and as a result of different gamete exposures (eggs, sperm, or both eggs and sperm, Table 1). In addition, we included a control group in which neither gamete was exposed to sunscreen.

Five 100 ml aliquots of the 5% dilution egg stock were exposed to the following five treatments: a control (no sunscreen), a high concentration of physical and chemical sunscreen (1ml sunscreen stock in 100 ml = 1% solution, 1:100 dilution), or a low concentration of physical and chemical sunscreen (100µl sunscreen stock in 100ml = 0.1% solution, 1:1000 dilution) (See table 1). Five aliquots containing 5 ml of a 1:250 dilution of sperm in FSW were prepared and exposed to the following five treatments: a control (no sunscreen), a high concentration of physical and chemical sunscreen (20µl sunscreen stock in 5ml = 0.4% solution,), and low concentration of physical and chemical sunscreen (2µl sunscreen stock in 5ml = 0.04% solution) concentration of chemical or physical sunscreen solution (See table 1). We did not perform all cross-tested combinations of eggs and sperm at high or low concentrations of sunscreens (eg. egg high, sperm low) because sea urchins are social broadcast spawners and gametes would experience similar conditions.

After 20 mins of exposure, 10ml of egg exposed to the respective treatments were aliquoted into vials and left for another 10 mins for a total exposure time of 30 mins. After 30 mins, various treatments of sperm were mixed with the eggs in the scintillation vials (1:50,000 dilution). After 10 mins, formalin (1%) was added to each vial to halt fertilization. Two hundred embryos were counted to calculate the percentage of eggs fertilized for each sample.

2.6 Data Analysis of Fertilization Assay

We assessed differences in the proportion of eggs fertilized using linear regression, fitting sunscreen type, gamete exposure, and the interactions between sunscreen type with exposure (six levels) and concentration with exposure (six levels) as predictors (hereafter referred to as the "full model"). Because the "high" and "low" concentrations differed between gametes, we did not include concentration as a main effect, and instead adjusted for the interaction between exposure and concentration. The proportion data were heteroskedastic (as is characteristic of data originating from a binomial process), so we logit transformed proportions before analysis (Warton & Hui, 2011), which fit the assumptions of a linear regression. Because some fertilization proportions were exactly 0 or 1, we shrank the proportion data to the interval [ε, 1 - ε] where ε = 0.01 (the smallest non-zero value in the data) to allow for logit transformation. The qualitative (significance test) results were identical for other intervals we tested, including ε = 0.001 and ε = 0.025.

We assessed differences between each treatment group and the control using a Dunnett's test (Dunnett, 1955; Hothorn, Bretz, & Westfall, 2008). Following the full model, we used a series of Student's t-tests to assess differences between 1) chemical and physical sunscreen at the same concentrations and 2) concentrations of the same sunscreen types. We adjusted for multiple comparisons using the Benjamini & Hochberg method for controlling the rate of false discovery (Benjamini & Hochberg, 1995; Pike, 2011). For all statistical tests, we considered values below a threshold of α = 0.05 to be statistically significant. We performed all analyses using R version 3.5 (R Core Team, 2018).

3. Results

3.1 Sperm dilution assay

Results from the sperm dilution assay were assessed to identify a target sperm concentration that would allow for successful fertilization in the control (minimum of 90% fertilized), while simultaneously allowing us to observe some effects of sunscreens. Based on our results, we determined the optimal dilution of sperm to be 1:50,000 (Figure S1).

3.2 Sunscreen Exposure Assay

On average, fertilization success was significantly lower for treatments in which gametes were exposed to chemical sunscreen than for treatments in which gametes were exposed to physical sunscreen, suggesting that chemical sunscreen is comparatively more detrimental to sea urchin fertilization (F(1, 87) = 11.44, p = 0.001, Table S1, Figures 1 & 2). Average fertilization success for the control (no sunscreen) was 97%, while average fertilization success across all gamete exposures and concentrations was 31% for chemical sunscreen and 52% for physical sunscreen (Figure 1). Fertilization success was significantly lower than the control for all treatment groups in which gametes were exposed to chemical sunscreen. In comparison, three treatment groups for which gametes were exposed to physical sunscreen were not significantly different from the control including: sperm at low and high sunscreen concentrations, and eggs at low sunscreen concentrations (Figure 1, Table S2). Overall, results from the full model and comparisons to control suggest that chemical sunscreen is more harmful to sea urchin fertilization than physical sunscreen. However, there was a significant interaction between sunscreen type and gamete exposure on fertilization success (F(2, 87) = 5.58, p = 0.005, Table S1), suggesting the effect of sunscreen type is gamete-specific.

For treatments in which sperm were exposed, the percentage of eggs fertilized was lower for chemical sunscreen than for physical sunscreen at both low (Δ = 25%) and high (Δ = 68%) concentrations. The percentage of successful fertilization when sperm were exposed to high or low concentrations of physical sunscreen did not differ significantly from the control, while fertilization was significantly lower for sperm exposed to either concentration of chemical sunscreen compared to the controls (Figure 1, Table S3). However, fertilization success was not significantly different between physical and chemical sunscreen at low concentration (p = 0.21,

Table S3). While fertilization success did not differ between low and high concentrations of physical sunscreen (Δ = 1%, $p = 0.96$), fertilization declined substantially at high concentrations of chemical sunscreen, resulting in a significant difference between chemical and physical sunscreen at high concentrations ($p < 0.001$).

For treatments in which eggs were exposed, results were similar to those of the sperm exposure at low concentration, but were notably different at high concentration. At low concentration, exposure to chemical sunscreen resulted in 21% lower fertilization than physical sunscreen, although this difference was not significant ($p = 0.28$). Consistent with results from the sperm exposure, fertilization success at low concentration of physical sunscreen did not differ significantly from the control ($p = 0.69$), while fertilization for chemical sunscreen at low concentration was significantly lower than the control ($p = 0.03$, Table S3). At high concentration, however, fertilization was significantly lower than the control for both sunscreens (Figure 1, Table S3). Although not significantly different, fertilization success was 21% higher for eggs exposed to chemical sunscreen than for those exposed to physical sunscreen at high concentration ($p = 0.28$).

Exposing both eggs and sperm to low concentrations of sunscreen resulted in a lower percentage of fertilized eggs overall, and while the difference in fertilization success between chemical and physical sunscreens was not significant, it was similar to that observed in egg and sperm exposures (Δ = 34%, $p = 0.12$). All groups for which both eggs and sperm were exposed to either sunscreen differed significantly from the control (Figure 1, Table S3), and when both gametes were exposed to high concentrations, eggs appeared abnormal and the fertilization rates were near zero for both sunscreens (Figures 1 &3).

In summary, at low concentrations in all exposure groups (eggs, sperm, and both), we observed between 21% and 34% lower fertilization success for chemical sunscreen compared to physical sunscreen (Figure 1), although these differences were not significant. These comparisons were likely not significant due to disparities in statistical power and sample size, because estimated differences in fertilization between chemical and physical sunscreen were similar between the full model and all exposures at low concentrations (Figure 2). Together, results from pairwise comparisons appear consistent with observations from the full model in suggesting that chemical sunscreen is more harmful to sea urchin fertilization at low concentration, and that high concentration of physical sunscreen may have a gamete-specific effect.

4. Conclusions

Our results indicate that both chemical and physical sunscreen can negatively affect the gametes and fertilization of sea urchins, but that physical sunscreen is potentially less harmful at lower, more environmentally relevant concentrations (Figure 1). Although there have been several studies on individual sunscreen toxicity, this study is the first to examine comparative effects of physical and chemical sunscreen on fertilization of sea urchin embryos. While some studies have shown that physical sunscreens can cause abnormal development in a variety of taxa (Hanigan et al., 2018; Sendra et al., 2017; Wong et al., 2010), effects of chemical sunscreens are more well documented (Danovaro et al., 2008; Downs, 2014; Downs, 2015; McCoshum et al., 2016), and little is known about their comparative effects. Our results are consistent with previous studies on the effects of chemical sunscreens, and demonstrate the potential harm of chemical sunscreens to the fertilization of temperate marine invertebrates (Corinaldesi et al., 2017; Coronado et al., 2008; Danovaro et al., 2008).

Comparatively, exposure to chemical sunscreen was in general more harmful to sea urchin fertilization than physical sunscreen (Figure 2). We observed ubiquitously lower fertilization success for gametes exposed to "low" concentrations of chemical sunscreen, regardless of gamete exposure (Figure 1). While the absolute concentrations of ingredients tested are unknown, differences in fertilization success observed at low concentrations are particularly relevant since they likely approximate sunscreen levels in local ecosystems.

We also documented that the higher concentrations of physical sunscreen had a gamete-specific effect. Eggs exposed to high concentrations of physical sunscreen experienced poor fertilization success (7%) compared to sperm exposed to a similarly high concentration of physical sunscreen (78%). In addition to lower fertilization success, we observed physical deformities of eggs in the egg exposure at high concentrations (10,000 ppm) of physical sunscreen (Figure 3b). While some of the deformed eggs had a partial fertilization envelope present, it is unlikely that any of the eggs would have developed normally. This phenomenon was also observed in a study by Kobayashi & Okamura (2004) where they exposed sea urchin gametes to zinc. In contrast, eggs exposed to high concentrations of chemical sunscreen did not show any deformity of the eggs, and had slightly higher fertilization success (Figure 3c). While in general physical sunscreen appears less harmful to sea urchin fertilization, our results provide some evidence that this effect may be gamete specific and concentration-dependent.

While it was beyond the scope of this project to determine the specific mechanism of damage by commercial sunscreens, it is clear that both eggs and sperm may be affected causing a synergistic decrease in fertilization. For example, Cunningham et al. (2020) demonstrated that

Zn can enter the embryos and decrease the function of ABC multi-drug resistance transporters. Therefore, multiple mechanisms might be affected by these sunscreens. More specifically, exposure of the eggs to these sunscreens may be affecting fertilization by damaging the glycoproteins (Resact) in the jelly or by simply removing the protective jelly coat and thus interfering with sperm attraction and the acrosomal reaction (Hansbrough & Garbers, 1981). These sunscreens might act directly on components of the jelly coat or potentially dissolve the jelly coat by lowering the pH of the seawater.

Sunscreen solutions may also be affecting other components of the eggs including the egg receptor for Bindin (ERB, Kamei et al., 2003) or rafts in the egg lipid surface containing the ERB (Belton et al., 2000), the vitelline envelope, cortical vesicles and activation of the eggs to elevate the fertilization envelope (Epel, 1975). All of these components are involved in the slow block to polyspermy, which involves binding of the sperm to the egg and subsequent activation of the PIP cycle, calcium release and release of the cortical vesicles and elevation of the fertilization envelope (Carroll et al, 1999; reviewed in Whitaker & Swann, 1993; Runft et al., 2002). Similarly, while metals such as zinc are neither basic or acidic, oxides are basic in nature and thus may have caused a change in membrane potential of the eggs (the fast block to polyspermy), creating conditions unconducive to fertilization (reviewed by Whittaker & Swann, 1993). Further studies examining whether sunscreens specifically affect molecular, structural or physiological components of the eggs will better elucidate the mechanisms of damage.

Similarly exposure of sperm to chemical sunscreen significantly affected fertilization, while physical sunscreen only affected sperm modestly. Therefore, the sunscreen solutions may be affecting different aspects of sperm efficacy. For example, sunscreens may be causing decreased sperm motility. As mentioned, egg jelly coats which attract sperm may be affecting sperm activity, but sperm are also particularly sensitive to pH levels and only become motile upon release into seawater at a pH of 7.6 (Christiansen et al., 1986). Therefore, any decrease in the pH could affect motility. In addition, sunscreen solutions may interfere with the sperm acrosome reaction, which releases enzymes that digest the jelly coat creating a path for sperm to get to the egg and which exposes Bindin in the membrane the sperm to be able to interact with the ERB on eggs (Vacquier, 1986, Vacquier & Hirohashi, 2004). Similarly, chemical sunscreens may act directly on the structure or activity of Bindin protein on the sperm. More extensive comparative studies of how sunscreens affect sperm activity at the molecular level will help us understand these mechanisms better.

Our study is among few to evaluate the impacts of sunscreens on temperate marine invertebrates. Abiotic conditions, including temperature, light, and nutrient cycling, differ substantially between temperate and tropical ecosystems, and there may be important differences in how sunscreens are metabolized by organisms in these ecosystems. Although physical and chemical sunscreens and their components negatively affect organismal health, the level of their toxicity may depend on a combination of factors. In a field study, Donavaro et al. (2008) observed synergistic effects of temperature and sunscreens on coral retention and membrane integrity of zooxanthellae, and noted that corals in warmer waters experienced higher rates of sunscreen-induced bleaching. Downs et al. (2009) observed the effects of oxybenzone on corals in both light and dark conditions and found that corals exposed to oxybenzone in light experienced direct injury to zooxanthellae, and corals exposed in dark experienced bleaching.

Toxicity of sunscreens are sometimes also due to the breakdown of their components. The toxicity of zinc-oxide, for example, results from the rapid breakdown of Zn into Zn^{2+} (Fairbairn et al., 2011; Kobayashi & Okamura, 2004; Manzo et al., 2013). Fairbairn et al. (2011)

examined iron-doping as a way to remediate the toxic effects of Zn, but found that iron-doped Zn is just as harmful to marine embryos as non-iron-doped Zn. Future studies that better identify the impact of the synergistic effects of sunscreens and the abiotic environment, such as temperature and light, could help us to better understand the impacts of sunscreens globally.

While more research is needed, the results of this study suggest that encouraging a shift in sunscreen usage from chemical to physical sunscreen types may lessen anthropogenic impacts on marine coastal ecosystems. As beach visitation and marine ecotourism continue to increase globally, research into alternative forms of sun protection may also be worth exploring as environmentally safer options (Wood, 2018). Past research has attempted to mimic natural sunscreens found in wildlife and use organic matter such as coffee grounds as a substitute (Dunlap et al., 1998; Marto et al., 2016), but despite such efforts, physical and chemical sunscreens still lead the market (EWG, 2018). Regardless, continued research into the effects of cosmetics and their specific ingredients on marine organisms is crucial to the mitigation of anthropogenic impacts in oceanic ecosystems.

Tables and Figures

Table 1. Our factorial design crossings among treatment (no sunscreen, physical or chemical sunscreens), concentration of sunscreens (high and low), and exposure of gametes (egg, sperm, both, control/neither) as our factors.

	None	**Physical Sunscreen**		**Chemical Sunscreen**	
		LOW	**HIGH**	**LOW**	**HIGH**
Control	Ø	-	-	-	-

Egg	-	♀L	♀H	♀L	♀H
Sperm	-	♂L	♂H	♂L	♂H
Both	-	♀L ♂L	♀H ♂H	♀L ♂L	♀H ♂H
	Egg (100ml)→ H: 1ml , L: 100μl | Sperm (5ml) → H: 20μl , L: 2μl				

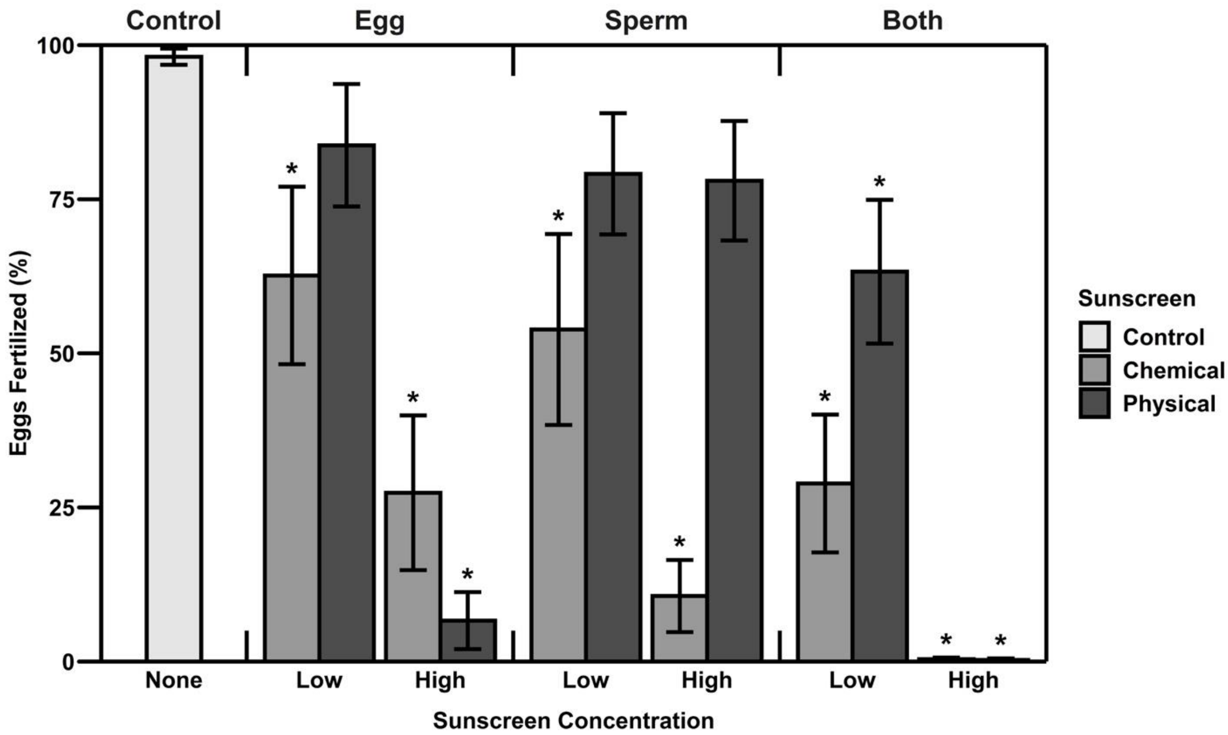

Figure 1. Average percent fertilization of *S. purpuratus* gametes for each sunscreen treatment type, sunscreen concentration and gamete exposure. Error bars represent ± standard error of the mean. Asterisks (*) indicate a significant difference in fertilization compared to the control ($p < 0.05$) according to a Dunnett's test (n = 8 per comparison).

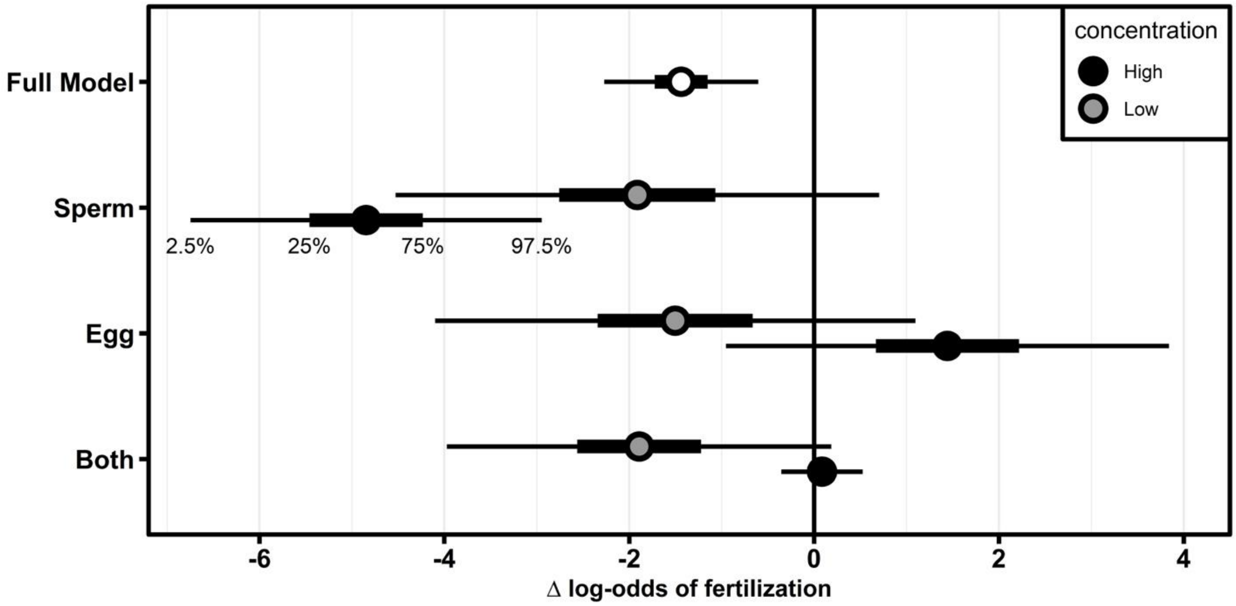

Figure 2. Difference in log-odds of fertilization between chemical and physical sunscreen, on average and for high and low concentrations for each exposure (sperm, eggs, and both). Results for the full model are averaged across other terms; other results are estimated using t-tests. Differences below 0 indicate lower fertilization success for chemical sunscreen compared to physical; results are given as 50% and 95% confidence intervals.

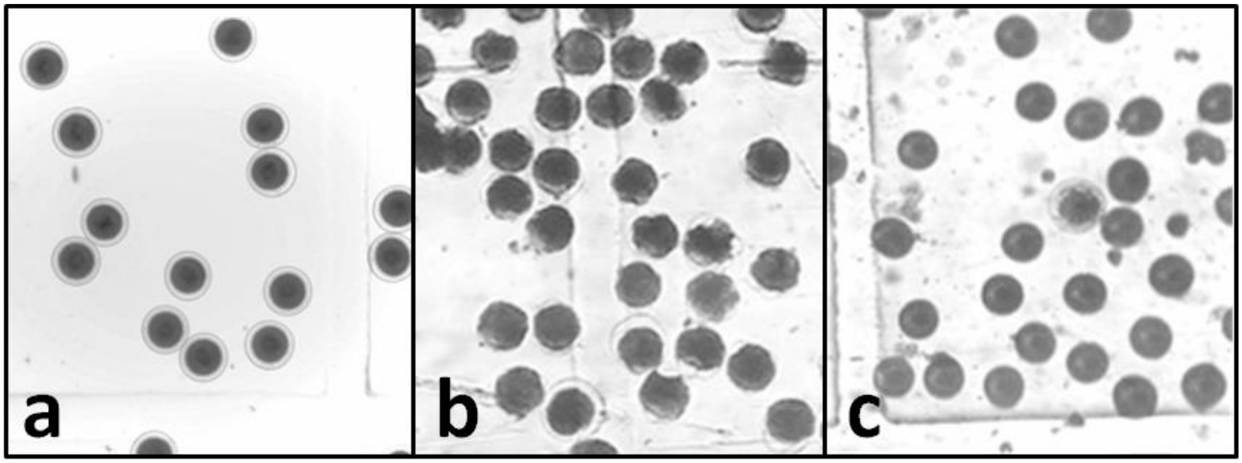

Figure 3. Post-fertilization images of *S. purpuratus* embryos where: a) eggs were not exposed to sunscreens with healthy fertilized eggs, b) eggs were exposed to the high concentration of physical sunscreen, and c) eggs were exposed to the high concentration of chemical sunscreen.

Declaration of Competing Interest

No conflicts of interest are declared by the authors.

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7. Supplemental Materials

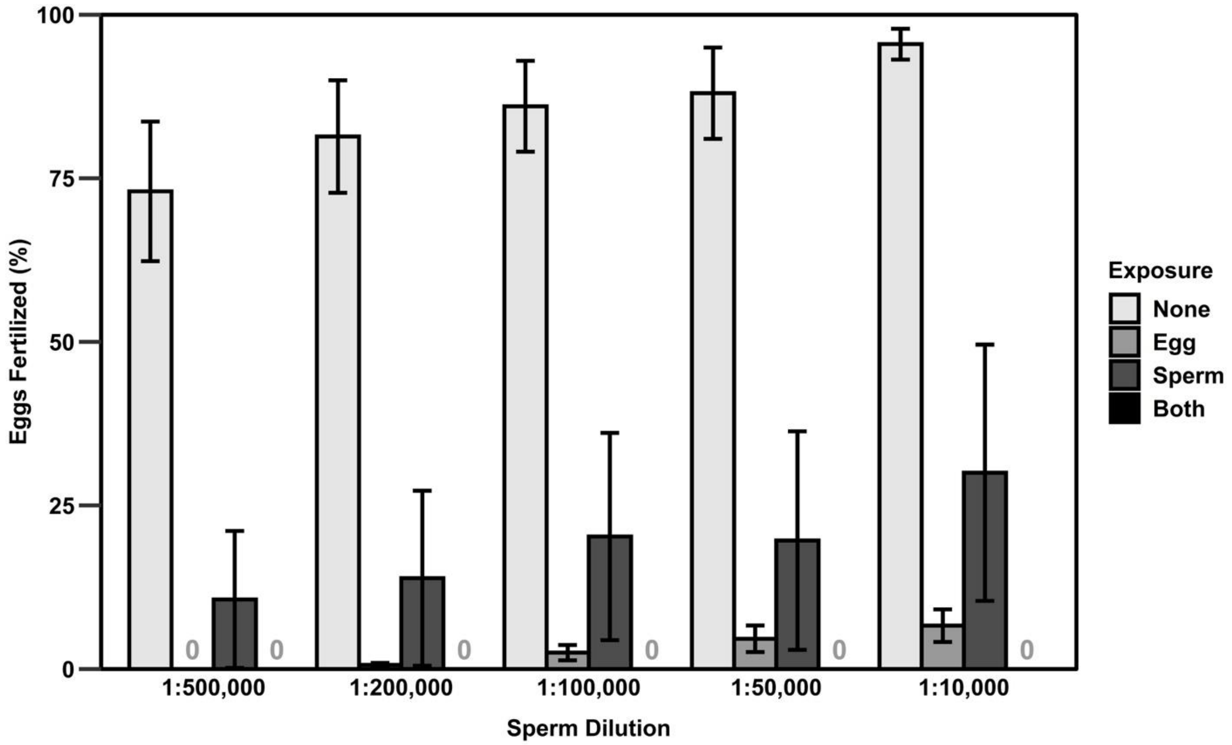

Figure S1. Percent fertilization of *S. purpuratus* gametes for each gamete-treatment type at different dilutions of sperm (sperm v:v FSW). Error bars represent ± standard error of the mean.

Table S1. Results of linear regression evaluating the effect of treatment (no sunscreen, physical or chemical sunscreen), exposure (egg, sperm, or both egg and sperm), and the interactions between treatment with exposure and exposure with concentration on fertilization rates in sea urchin, *S. purpuratus*. To examine gamete specific effects, we followed this model with pairwise comparisons, the results of which are presented in Table S3. P-values in bold are significant at the α = 0.05 level.

Term	**df**	**Sum Sq**	**Mean Sq**	**F**	**Pr(>F)**
treatment	1	49.54	49.55	11.44	0.001
exposure	2	111.89	55.94	12.92	< 0.001
treatment:exposure	2	48.32	24.16	5.58	0.005
exposure:concentration	3	339.96	113.32	26.17	< 0.001
Residuals	87	376.71	4.33		

Table S2. Results of Dunnett's test for comparisons to the control. Estimates are the difference in log-odds of fertilization between each group and the control. P-values in bold are significant at the α = 0.05 level.

Exposure	Sunscreen	Concentration	Estimate	SE	t	p
Both	Physical	High	-6.906	0.828	-8.344	< **0.001**
		Low	-2.565	0.828	-3.099	**0.024**
	Chemical	High	-6.864	0.828	-8.294	< **0.001**
		Low	-4.335	0.828	-5.238	< **0.001**
Egg	Physical	High	-6.313	0.828	-7.627	< **0.001**
		Low	-1.2	0.828	-1.45	0.687
	Chemical	High	-5.106	0.828	-6.169	< **0.001**
		Low	-2.504	0.828	-3.026	**0.029**

Sperm	Physical	High	-1.532	0.828	-1.851	0.396
		Low	-1.56	0.828	-1.884	0.375
	Chemical	High	-5.728	0.828	-6.921	< **0.001**
		Low	-3.209	0.828	-3.877	**0.002**

Table S3. Results of pairwise comparisons between a) chemical and physical sunscreens within the same exposure and concentration, and b) different concentrations of the same sunscreen and exposure. Means and standard errors are on the log-odds scale; p-values are adjusted for multiple comparisons using the Benjamini & Hochberg method. P-values in bold are significant at the α = 0.05 level.

a. Comparisons between chemical & physical sunscreen

Exposure	Concentration	Chemical mean	Physical mean	SE	t	df	p
Egg	Low	0.97	2.48	1.21	-1.24	14	0.283
	High	-2.26	-3.71	1.12	1.29	14	0.283
Sperm	Low	0.1	2.01	1.22	-1.57	14	0.209
	High	-2.79	2.06	0.89	-5.47	14	< **0.001**
Both	Low	-1.08	0.81	0.97	-1.95	14	0.122
	High	-4.37	-4.46	0.21	0.42	14	0.742

b. Comparisons between high and low concentrations of the same sunscreen

Exposure	Sunscreen	Low mean	High mean	SE	t	df	p
Egg	Physical	2.48	-3.71	0.93	6.63	14	< **0.001**
	Chemical	0.97	-2.26	1.36	-2.38	14	0.064
Sperm	Physical	2.01	2.06	0.97	-0.05	14	0.961
	Chemical	0.1	-2.79	1.15	-2.51	14	0.06
Both	Physical	0.81	-4.46	0.62	8.47	14	< **0.001**
	Chemical	-1.08	-4.37	0.77	-4.26	14	**0.002**