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Toxicological Assessment of Zinc Oxide and Titanium Dioxide on Staghorn Coral, Acropora cervicornis

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ABSTRACT

Coral reefs are one of the most diverse and biologically significant ecosystems on the planet. However, anthropogenic impacts have led to a global decline in overall reef health in recent years. To address concerns that organic ultraviolet filters in sunscreen may contribute to reduced coral health, the mineral UV filters, zinc oxide (ZnO) and titanium dioxide (TiO_2), have become more common in sunscreen formulas as they are often considered "reef safe" despite limited information on the toxicological effects of these compounds in corals. This study evaluated the effects of exposure to unmodified forms of zinc oxide or titanium dioxide compared to the commercial products ZinClear IM® 50CCT (ZnO 50%) and UV Cut (TiO_2 40%) on the threatened Atlantic scleractinian coral, *Acropora cervicornis*. Exposure-associated stress on coral fragments was determined utilizing measurements of photosynthetic efficiency, behavioral responses, and calcification in 48-h exposure assays. Corals exposed to TiO_2 were observed to have mild to moderate response during the 48 h exposure but ultimately returned to normal behavior at the end of the 48-h exposure. Corals exposed to ZnO had moderate to severe responses, with a high percentage of tissue mortality at concentrations > 190.7 µg/L. Exposure to ZinClearIM® 50CCT or UV Cut resulted in no significant response and no mortality. Corals had a significantly greater response to ZnO in comparison to TiO_2.

KEYWORDS

zinc oxide, titanium dioxide, toxicity, *Acropora cervicornis*, sunscreen, mineral filters

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INTRODUCTION

Coral reefs are some of the most biologically diverse ecosystems, providing habitat and resources in areas where nutrients are scarce (Dodge et al. 1984, Cole et al. 2008, Perry et al. 2013, Corinaldesi et al. 2018, Raffa et al. 2019, Mitchelmore et al. 2021, Watkins and Sallach 2021). These ecosystems have an important role in shoreline protection and function as critical habitats for marine organisms (Woodruff 2019, Sharifan 2020). Coral reefs are one of the oldest ecosystems on the planet, with the first signs of modern coral formation present during the Triassic period (Dubinsky and Stambler 2010). Corals can construct unique marine habitats through the secretion of calcium carbonate skeletons and the establishment of dense colonies on the benthos (Perry et al. 2013, Raffa et al. 2019). In oligotrophic tropical seas, hermatypic corals produce the foundation that allows a variety of organisms to survive, and (Knap et al. 1983, Dodge et al. 1984, Miller et al. 2021) approximately 1-9 million marine species rely on coral reefs as critical habitats for survival (Watkins and Sallach 2021).

Throughout geologic time, coral structures have withstood damage from natural threats and gradual climate shifts (Raffa et al. 2019). However, rapid and extreme temperature fluctuations and drastic environmental alterations can have devastating effects on reef ecosystems (Raffa et al. 2019). The health of a coral ecosystem is strongly correlated to the establishment and development of individual coral species (Cole et al. 2008). Reef health also influences the amount of income for a local economy with tourism providing opportunities for financial gain to the region, particularly coastal developments (Hu et al. 2003, Corinaldesi et al. 2018, Sharifan 2020, Miller et al. 2021, Mitchelmore et al. 2021, Watkins and Sallach 2021). The economic value of coral reefs to the surrounding countries is estimated to supply a revenue of between $30 – USD 375 billion to localized areas, which is especially true in coastal regions (Nepote et al. 2016, Raffa et al. 2019). In the Caribbean, tourism is the source of more than $USD 90 billion annually, largely due to the abundance of reef habitats. (Jovanoić and Guzmán 2014). However, increased tourism contributes to an increase in the level of anthropogenic impacts on reefs, which can cause a decline in the overall health of coral populations.

Marine environments are sensitive to an extensive assortment of anthropogenic stressors in subtropical and tropical oceans (Knap et al. 1983, Dodge et al. 1984, Brown 1997, Szmant 2002, Corinaldesi et al. 2018). In heavily urbanized areas, a shift in the overall health of an ecosystem can occur from an exponential increase in tourism and residency, which is partially due to

industries such as tourist resorts, aquaculture facilities, and various coastal services (Danovaro et al. 2008, Downs et al. 2014, Nepote et al. 2016, Eastwood et al. 2017, Watkins and Sallach 2021). These commercial additions to the localized areas typically have a negative effect on reef health including outputs such as waste discharge, run-off of nutrient-enriched waters, removal or degradation of mangrove and seagrass habitats, increased sedimentation, trash accumulation, and physical damage to corals from direct interactions with tourists (Eastwood et al. 2017). Anthropogenic activities have directly threatened approximately 58-70% of coral coverage in the Caribbean, leading to potentially permanent reef damage (Downs et al. 2005, Nepote et al. 2016). As these impacts persist, the growth of coral colonies could decline, triggering a collapse in coral reef infrastructure and ecosystem vitality (Perry et al. 2013).

Even at low concentrations, acute and chronic anthropogenic inputs can cause damage to coral species that are sensitive to these influences (Dodge et al. 1984, Castro and Huber 2013, Nepote et al. 2016). One of the most recognizable displays of physical distress and critical indicators of coral health is bleaching (Fel et al. 2019, Raffa et al. 2019). In corals, bleaching is described as the dissociation of the symbiotic zooxanthellae and/or pigment loss, yielding a pale or white appearance of the corresponding host (Brown 1997, Fel et al. 2019, Raffa et al. 2019, Sharifan 2020). Environmental stressors lead to the expulsion of zooxanthellae when photosystem II protein synthesis is inhibited and the process of carbon dioxide fixation is disrupted (Jovanoić and Guzmán 2014). Prolonged bleaching has the potential to cause partial or complete coral tissue mortality (Raffa et al. 2019). Coral death can occur in a short amount of time if zooxanthellae do not repopulate coral tissues after bleaching (Jovanoić and Guzmán 2014). Mass bleaching events have been linked to the decline of marine biodiversity and led to the destruction of coral reef habitats (Jovanoić and Guzmán 2014). These events can occur due to ocean warming, bacterial pathogens, excessive ultraviolet radiation, and/or the presence of land-based sources of pollutants and containments (Jovanoić and Guzmán 2014, Raffa et al 2019). Examples of potentially harmful chemicals include many of the active ingredients found in sunscreens, which are becoming increasingly detected in aquatic environments (Balmer et al. 2005, Danovaro et al. 2008, Corinaldesi et al. 2018, Johnsen 2018, Wood 2018, Casas-Beltran et al. 2020).

The manufacture and use of cosmetic sunscreens and personal care products have increased greatly due to the increasing awareness of the potential cancerous effects of ultraviolet A (UVA) and ultraviolet B (UVB) radiation (Moloney et al. 2002, Watkins and Sallach 2021). The vast

majority of skin-related cancers are caused by prolonged, chronic exposure to solar ultraviolet (UV) wavelengths that can lead to cell and DNA damage (Ngoc et al. 2019, Fournier et al. 2021). The European Union (EU) has established measurement techniques necessary for sunscreen products to become recognized as photo-protectants against harmful UV radiation (Lodén et al. 2011). For UV filtering chemicals to be sanctioned in sunscreen, the EU makes determinations based on studies of various exposure periods, genetic and reproductive toxicities, photoinduced skin irritations, and carcinogenic properties, in addition to the environmental aspects (Lodén et al. 2011).

These UV filters, which either absorb, reflect, or scatter UV light, include compounds such as benzophenone, octocrylene, octinoxate, homosalate, oxybenzone, titanium dioxide, and zinc oxide (Downs et al. 2016, Wood 2018, Ngoc et al. 2019, Schneider and Lim 2019, Levine 2020, NOAA 2021). Altering or modifying these ingredients has proven to be difficult due to the strict regulations set by the Scientific Committee for Consumer Safety (SCCS) for countries within the EU (Miller et al. 2021). This has limited the number of approved ultraviolet filters to twenty-nine chemical and mineral compounds due to the classification of sunscreen as an over-the-counter product (Miller et al. 2021). Currently, only sixteen compounds have been generally recognized as safe and effective (GRASE) UV filters by the U.S. Food and Drug Administration (FDA, Miller et al. 2021).

The concentration of UV filters differs between sunscreen products, as products may contain a combination of organic and/or inorganic compounds that are either listed as chemical or mineral-based (Wood 2018). Little is known about the long-term consequences of applying the chemicals found in sunscreen to the epidermis of the human skin, yet the promotion of daily sunscreen application has increased in recent years (Lodén et al. 2011, Alder and DeLeo 2020). Studies on mammalian species have indicated negative effects such as endocrine disruption, photo contact allergies, DNA damage, and the prevention of vitamin D formation when UV filters are absorbed through the skin (Lodén et al. 2011, Ngoc et al. 2019). In addition, studies that focused on human skin reactions have reported an increased risk of the development of melanoma in conjunction with prolonged sunscreen use (Ngoc et al. 2019). The FDA determined that 40 grams of sunscreen lotion per application is a safe and satisfactory quantity for protection from harmful UV radiation from the sun (Poiger et al. 2004, Giokas et al. 2007, Johnsen 2018, Watkins and

Sallach 2021). However, research has proven that consumers typically apply a significantly greater amount than is recommended per use (Giokas et al. 2007, Johnsen 2018, Casas-Beltran et al. 2020).

When sunscreen is applied to the skin, approximately 25% of the product application is washed off during recreational activities such as swimming and sunbathing (Daughton and Ternes 1999, Danovaro et al. 2008, Wong et al. 2010, Johnsen 2018, Casas-Beltran et al. 2020). These activities permit UV filters to directly enter freshwater and marine ecosystems, adding to the existing discharge from wastewater treatment effluents (Danovaro et al. 2008, Wong et al. 2010, Osmond and Mccall 2010, Johnsen 2018, Wood 2018, Casas-Beltran et al. 2020). Sunscreen components have been detected in 95% of wastewater and 86% of surface water globally (Balmer et al. 2005, Casas-Beltran et al. 2020). Recent studies have reported chemical and mineral sunscreen concentrations ranging between < 2 ng L^{-1} to 19 μg L^{-1} in freshwater lakes (Balmer et al. 2005, Casas-Beltran et al. 2020). In various Swiss lakes, chemical and mineral sunscreen concentrations were found to be higher during the warmer months, which correlated with the influx of tourists to the local area (Balmer et al. 2005). This volume of sunscreen waste results in significant potential for chemical contamination, degradation, and bioaccumulation of these compounds (Danovaro et al. 2008, Lodén et al. 2011, Johnsen 2018, Wood 2018, Mitchelmore et al. 2021).

The majority of sunscreen products contain a significant percentage (approximately 70 – 80% of the entire product) of the UV filters oxybenzone and/or octinoxate due to their unique chemical properties, which provide broad-spectrum protection (Raffa et al. 2019). Oxybenzone and octinoxate absorb UVA and UVB radiation by creating a photochemical reaction that reduces DNA damage to the skin (Chen et al. 2018, Barone et al. 2019, Raffa et al. 2019). When this reaction occurs, wavelengths emitted from the sun return the photon energy to its ground state (Raffa et al. 2019).

However, recent research has demonstrated that exposure to these UV filters may result in coral damage and mortality (Downs et al. 2016, Raffa et al 2019, Levine 2020). The UV filter known as benzophenone-2 (BP-2), a derivative of oxybenzone (benzophenone-3), has been listed as an emerging contaminant of concern and has been documented to cause a bleaching response in various stony corals (Downs et al. 2014, Downs et al. 2016, Wood 2018, Levine 2020, Agawin et al. 2022). Benzophenone-2 and various derivatives of the compound have the potential to induce DNA damage and increase the risk of genetic mutation (Lelièvre et al. 2007, Downs et al. 2016,

Wood 2018, Levine 2020). In particular, these compounds have been observed to have an adverse effect on coral planulae (Lelièvre et al. 2007, Downs et al. 2016, Wood 2018, Levine 2020). The persistence of UV filters in marine ecosystems may also leave coral colonies susceptible to infection and increase the likelihood and occurrence of bleaching events (Levine 2020).

Estimates of the quantity of sunscreens released into coral reef ecosystems range from 6,000 to 14,000 tons annually, and at least 40% of coastal reefs could be at risk of chronic exposure (Downs et al. 2016). Harmful effects from sunscreen components have not only been observed in coral species but other primary producers as well, possibly leading to bioaccumulation throughout the marine food web (NOAA 2021, Agawin et al. 2022). In a study on Mediterranean seagrasses, Agawin et al, (2022) reported that rhizome segments displayed higher concentrations of UV filters in areas of close proximity to urbanized regions, and concluded that UV filters have been accumulating within the seagrass for at least two decades. Barone et al. (2019) exposed clownfish to sunscreens containing oxybenzone over a 97-h treatment period. The fish were observed to display negative effects concerning feeding and swimming behaviors that lessened once the fish were removed from the dosing chambers (Barone et al. 2019). In chronic exposures, anemonefish displayed negative alterations in growth parameters such as body mass, length, and width along with signs of endocrine disruption when exposed to sunscreens at early developmental stages (Chen et al. 2018, Barone et al. 2019). This anti-androgenic activity can have lasting impacts on fish populations by lowering reproductive success and fitness within the species (Chen et al. 2018). Biomagnification and bioaccumulation can also lead to developmental deficiencies, endocrine disruption, impaired photosynthetic processes, promotion of viral infections, and decreased metabolic function (Chen et al. 2018, Barone et al. 2019, NOAA 2021, Agawin et al. 2022).

In an attempt to protect coral ecosystems from further damage, sunscreen products containing oxybenzone and octinoxate have been banned from all islands within the state of Hawaii effective in 2021 (Barone et al. 2019, Ngoc et al. 2019, Levine 2020, Miller et al. 2021, Agawin et al. 2022). In 2023, the Hawaiian legislature will also ban the distribution and sale of avobenzone and octocrylene (Relating to Water Pollution, 2021). Subsequently, locations such as Aruba, Palau, Marshall Islands, Bonaire, Mexico, and the U.S. Virgin Islands have begun to construct similar restrictions on these products and other UV filters (Levine 2020, Miller et al. 2021, Mitchelmore et al. 2021, Agawin et al. 2022). With increasing public awareness of the possible toxicological effects of sunscreen products on marine ecosystems, the cosmetic industry

has shifted focus to products that have an ecologically safer footprint, and are appealing to a more environmentally conscious market (Woodruff 2019).

Purportedly eco-friendly sunscreen products with “reef-safe” or “coral-safe” denotations have been advertised throughout the cosmetic market (Woodruff 2019, Miller et al. 2021). Products that display this claim increasingly contain zinc oxide and titanium dioxide due to their mineral-based classification by the FDA (Corinaldesi et al. 2018, Adler and DeLeo 2020, Fournier et al. 2021). Zinc oxide and titanium dioxide were approved as effective and safe ingredients in all application forms during the regulation changes in 2019 (FDA 2019).

Zinc oxide (ZnO) is a white semi-conductive inorganic compound that is found most commonly in crystalline formations when used in sunscreens and other cosmetic products (Kolodziejczak-Radzimska and Jesionowski 2014). In its various forms, this material has unique and versatile properties, which include high photostability, a broad range of radiation absorption, and a high electrochemical coupling coefficient (Kolodziejczak-Radzimska and Jesionowski 2014). Titanium dioxide (TiO_2) is also an inorganic white solid, which is typically found in polyforms anatase, brookite, and rutile (Kang et al 2019, Fournier et al. 2021). When TiO_2 is used for pigmentation, it is referred to as titanium white (Kang et al 2019, Fournier et al. 2021). Zinc oxide and titanium dioxide have high melting and boiling points and are water-insoluble (Kolodziejczak-Radzimska and Jesionowski 2014, Kang et al 2019).

Zinc oxide and titanium dioxide are beneficial in sunscreen products due to their photocatalytic interactions with ultraviolet radiation (Wormington et al. 2017). These two ultraviolet filters function as photoprotectants, absorbing UV radiation while permitting the reflection and refraction of UV photons (Schneider and Lim 2019). The most efficient UV protection is achieved when ZnO and TiO_2 are combined within the same product (Newman et al. 2009, Schneider and Lim 2019). This broad-spectrum protection against ultraviolet radiation and resultant limited penetration into the epidermis of the skin are some of the characteristics that have appealed to the cosmetic industry (Newman et al. 2009, Corinaldesi et al. 2018).

When mineral sunscreens were first manufactured, products containing ZnO and TiO_2 had thick or chalky formulas making the products cosmetically unappealing to consumers (Newman et al. 2009, Kolodziejczak-Radzimska and Jesionowski 2014, Schneider and Lim 2019, Alder and DeLeo 2020). The “bulk form” of the mineral ingredients did not blend with the skin as effortlessly as traditional sunscreens and left a white film upon application (Newman et al 2009, Schneider

and Lim 2019). Even though these larger particles are considered a safer alternative due to the lack of penetration into the skin, the industry changed to formulas that most appealed to the cosmetic market (Miller et al. 2021). These new products utilized nanosized particles of ZnO and TiO_2 that blended better with skin pigmentations, were less viscous, and were more transparent than the "bulk forms" (Newman et al. 2009, Kolodziejczak-Radzimska and Jesionowski 2014, Schneider and Lim 2019, Fournier et al. 2021).

Nanoparticles (or ultrafine particles) are defined as matter that is less than 100 nm with three Cartesian dimensions and cannot be detected under conventional microscopes, while "bulk particles" are categorized as matter > 100 nm (Gulson et al. 2010, Schneider and Lim 2019). Advantages of nanoparticles in sunscreen include a non-greasy formula that is inexpensive and transparent along with the lack of degradation when exposed to UV radiation (Schneider and Lim 2019). Nanoparticles exist in three common physical states: primary particles, agglomerates, and aggregates (Schneider and Lim 2019). Aggregates are the most common physical manifestation, especially in sunscreens with mineral ingredients ranging in sizes from 30 – 150 nm (Schneider and Lim 2019). Aggregates are formed when primary particles bind through physical and chemical properties while in suspension, leading to the formation of agglomerates when clumped (Schneider and Lim 2019). Nanoparticles now compose 30% of zinc and 70% of titanium compounds in sunscreen products, raising concern about the potential toxicity both in humans and in marine environments (Newman et al. 2009).

The FDA regulates nanoparticles to a certain degree but does not differentiate between smaller particles less than 100 nm (Osmond and Mccall 2010, Schneider and Lim 2019). The small size of these particles results in greater surface reactivity and larger surface areas per unit mass that could potentially infiltrate the human body's natural defense systems (Newman et al. 2009, Sharma et al. 2012). Zinc oxide and TiO_2 nanoparticles have the potential to penetrate through the human epidermis, which could lead to biological disruptions throughout the body (Newman et al. 2009, Sharma et al. 2012). In sunscreens, nanosized particles can possibly avoid the human body's natural defenses, generate complications within proteins, and stimulate the formation of free radicals (Newman et al. 2009, Sharma et al. 2012, Tang et al. 2017).

Zinc oxide and TiO_2 nanoparticles are also capable of generating electricity in photovoltaic cells, emitting electrons in the presence of ultraviolet light (Newman et al. 2009, Lewicka et al. 2013, Tang et al. 2017, Wood 2018). This reaction has the potential to form reactive oxygen

species (ROS) causing possible damage to DNA, lipids, and proteins in marine organisms (Newman et al. 2009, Miao et al. 2010, Sharma et al. 2012, Johnsen 2018). Reactive oxygen species are a collection of reactive molecules, free radicals, and oxygen-derived ions that can cause damage to cells, potentially leading to mortality in an "oxidative stressed" environment (Newman et al. 2009, Miao et al. 2010, Sharma et al. 2012, Wood 2018, Fournier et al. 2021). These chemically reactive species are extremely harmful to marine organisms, particularly at high ionic concentrations, and have been specified as "reef toxic" by The National Oceanic and Atmospheric Administration (NOAA) (Sharma et al. 2012, Wood 2018, NOAA 2021).

Manufacturers have attempted to combat the formation of ROS and reduce semiconductor products by using nanoparticles coated in magnesium, silica, or aluminum (Sharma et al. 2012, Wood 2018, Alder and DeLeo 2020). In experimental lab settings, coated nanoparticles have lower ROS production and prevented photocatalytic events when using products containing ZnO and TiO_2 (Sharma et al. 2012, Lewicka et al. 2013, Wood 2018, Schneider and Lim 2019). However, this technique only mitigated the issue by limiting the amount of ROS released into marine environments (Fournier et al. 2021). With limited knowledge of the interactions of these chemicals in marine ecosystems, more research is needed to understand potential harm. (Wong et al. 2010, Jovanoić and Guzmán 2014, Tang et al. 2017, Corinaldesi et al. 2018, Watkins and Sallach 2021). Given the global decline in the health of coral reefs and the steady rise in ocean temperature, all additional impacts on marine ecosystems need to be understood, to mitigate further damage to an already fragile ecosystem.

SIGNIFICANCE OF WORK

Corals are vital to the health of marine ecosystems and serve as environmental indicators of stress caused by anthropogenic influences, which is especially true when in close proximity to substantial coastal developments. The chemicals within sunscreen products have been listed as compounds of emerging concern and a complete assessment of the toxicity of these UV filters is required for comprehensive decisions on environmentally safe levels. This research seeks to address the knowledge gap that exists on the potential effects of sunscreen products on coral reef ecosystems. This study evaluated the toxicological impacts of unmodified ZnO, TiO_2, and two commercial sunscreen formulations on the Atlantic staghorn coral (*Acropora cervicornis*). Coral response was assessed based on mortality, changes in physical condition, photosynthetic

efficiency, and growth rate. In addition, sublethal concentrations (EC_{50}) were based on changes in coral condition, and acute effects (LC_{50}) were determined based on percent mortality in relation to measured concentrations. Determining the impact thresholds of these compounds and their commercial modified forms provides quantitative data that can support environmentally conscious choices in regulatory decision-making.

METHODOLOGY

Acclimation

Branch tips of *A. cervicornis* (4 – 6 cm) were collected from the NSU Onshore Coral Nursery. This coral species is ideal for testing due to its ecological importance in reef ecosystems and high success rate of survivorship post fragmentation (Dodge et al. 1984, Wong et al. 2010, Griffin et al. 2012, Schopmeyer et al. 2017, Tang et al. 2017). In addition, *A. cervicornis* has been proven most sensitive to anthropogenic stressors in toxicological assays, producing conservative sublethal and lethal endpoints when compared to other coral species (Turner et al. 2021). The coral branch tips were attached to ceramic tiles (2.5x2.5 cm dimensions, 0.25 cm thickness) using cyanoacrylate gel glue (Loctite® Super Glue Gel). Coral fragments were acclimated in a 300-gallon laboratory coral culture system at 26 °C for 2-3 weeks to allow for establishment and stabilization in a lab environment post fragmentation. Artificial seawater (35 PSU) was made using TropicMarin sea salt and reverse osmosis water. Light was provided by Ecotech Radion X4 Pro LED lights, programmed to provide day and night photoperiods similar to the environmental conditions in southern Florida. During the acclimation period, corals were fed coral-specific dissolved nutrients (Brightwell CoralAmino) three times per week. A primary assessment of the coral fragments was conducted during this time to establish a baseline condition of the fragments for comparison to the exposed corals later in the study.

Exposures

Effects of exposure to different concentrations of mineral UV filters were evaluated with 48-h static renewal exposure assays (Figure 1). Eight treatments were used in each assay, with three replicate exposure chambers per treatment and two coral fragments per replicate. Exposure chambers were randomly assigned to treatments and corals were randomly assigned to replicates.

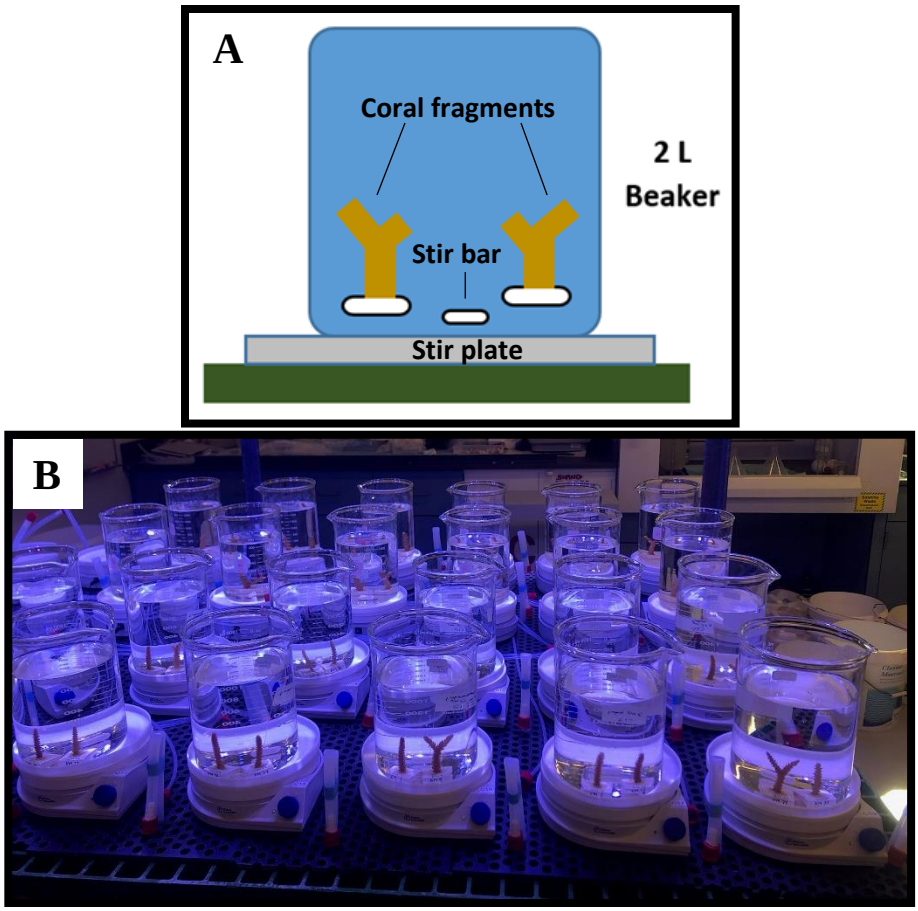


Figure 1. A) Illustration of experimental replicate B) Zinc oxide exposure system.

At the start of the exposure, before the coral fragments are added to the beakers, 1.5 L of filtered artificial seawater was added to each exposure chamber, and a magnetic spin bar was placed off-center to allow for the mixing of the solution without interfering with the coral fragments. Immediately after the corals were placed in the beaker, a specific amount of ZnO, TiO_2, or sunscreen product was added. The exposure media was renewed after 24 h; corals were first removed from each beaker, the exposure chambers were refilled with clean seawater, fragments were replaced, and the toxicant was added as previously described. This was conducted in increasing order of concentration to lessen potential contamination, beginning with the controls followed by the sunscreen products, and then the pure compound.

The first test examined the effects of zinc oxide (ZnO; powder; Sigma-Aldrich; > 97%; CAS No. 1314-13-2) compared to the commercial product ZinClear IM® 50CCT (ZnO 50%, Deveraux Specialties - Antaria). Eight treatments were used, including four concentrations of ZnO

(1000 µg/L, 500 µg/L, 250 µg/L, and 125 µg/L), three concentrations of ZinClear IM® 50CCT (1000 µg/L, 500 µg/L, and 250 µg/L), and a negative control (seawater only). Zinc oxide was weighed, pre-dissolved in 2 mL of DI water, and added directly to each exposure chamber. The ZinClear IM® 50CCT product was weighed onto glass slides, and the sides were placed upright in each exposure chamber. Slides and products were replaced when the exposure media was renewed after 24 h. The ZinClear treatment concentrations are expressed in terms of the concentration of the active ingredient, ZnO.

The second assay examined the effects of titanium dioxide (TiO_2; powder; Sigma-Aldrich; 99%; CAS No. 13463-67-7) compared to the commercial UV Cut (TiO_2-40-W; 40% dispersion of ultrafine TiO_2, Grant Industries, Inc.). Eight treatments were used, including four concentrations of the titanium dioxide (10 mg/L, 5 mg/L, 2.5 mg/L, 1.25 mg/L), three concentrations of UV Cut (10 mg/L, 5 mg/L, 2.5 mg/L), and a negative control (seawater only). Titanium dioxide was weighed, pre-dissolved in 2 mL of DI water, and added directly to each exposure chamber. The UV Cut product was weighed on glass slides, which were then placed upright in each of the exposure chambers. Product and slides were replaced after 24 h when the solutions were renewed. The UV Cut treatment groups were derived from the amount of the compound within each of the product formulations in terms of TiO_2. Test exposure concentrations were also based on the limited scientific literature where the dosing concentration was observed to cause a significant response in coral condition and/or mortality (Jovanoić and Guzmán 2014, Tang et al. 2017, Corinaldesi et al. 2018, Fel et al. 2019).

Concentrations of ZnO were measured with an Agilent 7500 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) utilizing helium in the collision cell to reduce potential inferences. Samples were collected in 50 mL polypropylene (PP) tubes from each of the beakers at the starting point (0 h), midpoint (before and after the test solution renewal), and the end of the exposures (48 h). Samples were vacuum filtered through a 0.45 Whatman cellulose acetate filter paper to separate particulates, then the filtrate was preserved with nitric acid (0.5% by volume) and stored at 4 °C. The cellulose filters were placed in individual 47 mm plastic Petri dishes and stored at -18 °C. Samples were sent overnight to the Chesapeake Biological Laboratory at the University of Maryland Center for Environmental Science for analysis.

The ICP-MS utilized internal standards to identify and measure the matrix of each sample to quantifying detectable compounds, specifically the concentration of ZnO. Filters were placed in a 20 mL quartz reaction vessel with 5 mL of concentrated nitric acid and left for 2 h to allow penetration to occur. Then, 5 mL of deionized (DI) water was added to the vessel and a vial containing the filter paper was covered with a quartz cap to be placed inside a Teflon cup. An additional, 5 mL of 30% hydrogen peroxide and 5 mL of ultrapure water was added before being capped and sealed. The reaction vessels were placed in the Milestone ETHOS Easy Advanced Microwave Digestion System for digestion and heated to 180 °C for 20 minutes. Samples were then refluxed at the same parameters and diluted with 15 mL of DI water once cooled. A subsample of this digestion (50-500 µL) was diluted to 10 mL and run through the ICP-MS for analysis.

Coral Evaluations

The physical condition of coral fragments during the exposure was semi-quantitatively scored using diagnostic criteria described in Renegar and Turner (2021) and Turner et al (2021). Briefly, each coral was scored based on five visual characteristics of coral stress, including tissue swelling, tissue attenuation, polyp extension/retractions, coloration, and mucus production with a score ranging from 0 (normal) to 3 (severe alteration). Corals were scored at the start of the exposure (0 h), 2 h, 6 h, 12 h, 24 h, 36 h, and at the end of the exposure (48 h). Detailed descriptions of the scoring matrix can be found in Table 1.

When corals were scored for condition alterations, observations were conducted to determine if any tissue exhibited lesions and/or tissue mortality. Corals with mortality were given an estimated percentage of tissue death in increments of 5%. Final mortality percentages were taken from the raw data to determine the mean mortality in each dosing beaker and the mean percentage for each treatment.

Table 1. Coral condition diagnostic with scoring range (table reproduced from Renegar and Turner 2021 and Turner et al. 2021).

Diagnostic Criteria	Range
Color	• **0 (normal):** color appears normal • **1 (mild):** slight lightening of coloration • **2 (moderate):** moderate lightening of coloration • **3 (severe):** significant lightening of coloration, evident bleaching
Polyps	• **0 (normal):** fully extended or loosely retracted • **1 (mild):** retracted and slightly closed • **2 (moderate):** evident polyp retraction with full polyp closure • **3 (severe):** polyps tightly retracted
Tissue swelling	• **0 (normal):** no swelling • **1 (mild):** slight coenenchyme swelling and/or polyp distension • **2 (moderate):** moderate coenenchyme swelling and/or polyp distension • **3 (severe):** severe swelling of coenenchyme and/or polyp distension
Tissue attenuation	• **0 (normal):** no attenuation • **1 (mild):** slight thinning of coenenchyme, flattening of polyps • **2 (moderate):** moderate thinning of coenenchyme and polyp flattening • **3 (severe):** severe tissue thinning, skeletal ridges exposed
Mucus production	• **0 (normal):** normal mucus production; no mesenterial filaments apparent • **1 (mild):** slightly elevated mucus production, no mesenterial filaments apparent • **2 (moderate):** moderately elevated mucus production; mesenterial filament extrusion possible • **3 (severe):** mucus sheets evident; possible mesenterial filament extrusion

Photosynthetic efficiency of the zooxanthellae was assessed with a Diving-PAM (Pulse Amplitude Modulated) fluorometer in dark conditions to obtain the dark-adapted maximum quantum yield (F_v/F_m, Ralph et al. 2016). Measurements were obtained after corals were kept in darkness for at least 60 minutes to allow for functional photosystem II (PSII) to oxidize following the reduction of the primary electron acceptor of PSII. This procedure exposes chlorophyll-α pigments to a saturation pulse of greater intensity that increases the irradiance, which significantly increases the fluorescence yield to a maximum (F_m) level, closing the PSII reaction center (Ralph et al. 2016, Turner et al. 2021). The difference between the minimum and maximum equates to the variable fluorescence (F_v), which can be used to produce a ratio to measure the efficiency of photochemistry (Ralph et al. 2016, Turner et al. 2021). The Diving-PAM aids in quantifying this ratio by measuring the maximum potential quantitative yield of PSII (F_v/F_m) in a localized area of the coral (Ralph et al. 2016, Turner et al. 2021).

The parameters used to measure dark-adapted maximum quantum yield in *A. cervicornis* were: measuring saturation intensity = 7, saturation width = 0.8, light intensity = 3, damping = 2, and gain = 3. (Ralph et al. 2016, Turner 2016). The combination of these values was established from the parameters of previous projects in conjunction with achieving a properly shaped fluorescence curve (*i.e.* flat topped) for effective measurement of the maximum potential quantitative yield of PSII. Two measurements of each coral were collected around the circumference of the branch tip, on opposite sides of the coral to avoid interference from the first measurement pulse. Measurements were taken directly before the start of the exposure and immediately after the 48 h period for fragments with measurable amounts of tissue post-exposure.

Buoyant wet weight was used to determine the growth rate of each coral fragment. This procedure measures the calcification of the fragment that occurs over a short amount of time and does not cause damage to the individual coral. The growth rate of the coral was determined from the loss or gain per day (mg/d) throughout the trial to evaluate potential inhibition of growth resulting from the exposure (Davies 1989). Growth rates were normalized from the initial fragment size and expressed as percent change per day (Ferrier-Pages et al. 2000). Assessments were conducted immediately before the initiation of the exposure and at the end.

Water Quality

Water quality samples were collected from each exposure chamber at the end of the exposure (48 h) once all corals were removed from the beakers. The concentration of nitrite (NO_2), nitrate (NO_3), ammonia (NH_3), and phosphate (PO_4) were determined with a HACH DR850 colorimeter. Alkalinity was measured by potentiometric titration with a Mettler-Toledo DL22 autotitrator. Temperature, pH, and dissolved oxygen (DO) were measured with a YSI 556 Multiprobe System.

Statistical Analysis

Data was analyzed using the statistical software R (Version 3.6.3) to determine the significance of the measured variables and exposure parameters. Data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test), and non-parametric tests were used when data did not meet assumptions of normality or homoscedasticity. Water quality parameters, photosynthetic efficiency, buoyant wet weight, mean coral condition, and mortality

were compared with a one-way analysis of variance (ANOVA, α=0.05) or Kruskal-Wallis analysis of variance on ranks (α=0.05). Post-hoc analyses were utilized when significant differences in treatment groups were present; Tukey's HSD Post Hoc Test was used for parametric data and Multiple Comparisons was used for nonparametric data. Sublethal and lethal thresholds were determined using the *drc* package in R. Coral scores were divided by the maximum possible score of 12 (Table 1) to obtain the percent effect on each coral, and then averaged for the two coral fragments in the beaker. The sublethal concentrations (EC_{50}) were calculated from mean coral condition in relation to the measured concentration in each of the corresponding beakers using a log-logistic 4-parameter dose-response model with maximum effect fixed at 1 (Renegar and Turner 2021, Turner et al. 2021). Lethal concentrations (LC_{50}) were calculated with a log-logistic 2-parameter dose-response model (Turner et al. 2021). Estimates for the 95% confidence intervals were represented utilizing the effect dose function in R, along with the delta method for all dose curves. Treatments that did not have measurable effects and/or presence of mortality were not run through the *drc* package. Metrics that had no observed response throughout the exposure were not analyzed.

RESULTS

Zinc Oxide Exposure

Water Quality

A summary of the water quality data is provided in Table 2. There were no significant differences (Kruskal-Wallis ANOVA, $p > 0.05$) in the water quality parameters at the start of the exposure between the treatment groups and the control. Post-exposure, DO was significantly greater in the 1000 µg/L ZnO treatment replicates compared to the controls ($p = 0.020$). Phosphate was significantly higher in the 250 µg/L ZnO, 500 µg/L ZnO, and 1000 µg/L ZnO replicates in comparison to the controls ($p = 0.032$, $p = 0.001$, and $p = 0.019$). The ZinClear IM® 50CCT replicates had a significantly higher pH in the 500 µg/L ZinClear and 1000 µg/L ZinClear treatments compared to the control ($p = 0.021$ and 0.035). The highest concentration of ZinClear (1000 µg/L) also had a significantly higher PO_4 ($p = 0.041$) compared to the control. No significant differences were found for temperature (°C), NO_2, NO_3, and NH_3 ($p > 0.05$) between any of the ZnO and ZinClear IM® 50CCT treatments compared to the control groups.

Table 2. Mean water quality (± SD) parameter at 48 h for ZnO and ZinClear IM® 50CCT. Samples denoted with (~) did not have the measurements needed for an accurate reading.

Treatment	Temp (°C)	pH	DO (ppm)	Alk (ppm)	PO_4 (ppm)	NH_3 (ppm)	NO_2 (ppm)	NO_3 (ppm)
Control	23.6 (±0.06)	8.12 (±0.01)	7.1 (±0.05)	122 (±5.82)	0.33 (±0.02)	0.00 (±0.05)	0.01 (0.00)	0.01 (0.00)
Zinc oxide								
125 µg/L	23.7 (±0.10)	8.14 (±0.02)	7.2 (±0.01)	127.4 (±2.98)	0.27 (±0.03)	0.01 (±0.01)	0.01 (0.00)	0.01 (0.00)
250 µg/L	23.6 (±0.12)	8.14 (±0.02)	7.1 (±0.01)	125 (±0.85)	0.36 (±0.09)	0.01 (±0.00)	0.01 (0.00)	0.01 (0.00)
500 µg/L	23.5 (±0.00)	8.15 (±0.01)	6.8 (±0.28)	128 (~)	0.50 (±0.29)	0.01 (±0.07)	0.01 (0.00)	0.01 (0.00)
1000 µg/L	23.5 (±0.06)	8.13 (±0.01)	6.7 (±0.16)	134 (~)	0.43 (±0.06)	0.01 (±0.01)	0.01 (0.00)	0.01 (0.00)
ZinClear IM ® 50 CCT								
250 µg/L	23.5 (±0.06)	8.12 (±0.01)	7.2 (±0.07)	121 (±3.39)	0.27 (±0.03)	0.01 (±0.00)	0.01 (0.00)	0.01 (0.00)
500 µg/L	23.5 (0.06)	8.15 (±0.01)	7.3 (±0.06)	120 (±0.18)	0.27 (±0.01)	0.01 (±0.01)	0.01 (0.00)	0.01 (0.00)
1000 µg/L	23.5 (±0.06)	8.16 (±0.01)	7.2 (±0.04)	121 (±8.13)	0.29 (±0.02)	0.01 (±0.01)	0.01 (0.00)	0.01 (0.00)

Exposure Concentrations

The concentrations measured in each chamber at each time point are provided in Table 3. All of the treatment means were considerably lower than the nominal concentrations, especially the treatments from the ZinClear IM® 50CCT concentrations. However, this was to be expected due to the insolubility of these compounds in seawater and the lipophilic nature of these compounds (Fel et al. 2019). The ZinClear IM® 50CCT product was also manufactured as a paste or cream making the compound less available in the water column, which yields a lower concentration. Measurements that were recorded below the detection limit were listed as BD in the

table below. In addition, the coefficient of variation indicates that the measurements have a high variability and are distant from the mean.

Table 3. Measured concentrations of ZnO (µg/L) in each exposure chamber over time (BD = below detection); the coefficient of variation was included with the listed treatment mean.

Treatment	T0	T24	T24P	T48	Mean (±CV)	Treatment Mean (±SE)
Control	3.9	0.0	0.0	0.0	1.0 (± 200%)	
	0.4	0.0	0.0	0.0	0.1 (± 200%)	
	0.4	0.0	0.0	0.0	0.0 (± 200%)	0.4 (± 0.21)
Zinc Oxide						
125 µg/L	25.6	44.4	18.8	11.7	25.1 (± 56%)	
	36.6	82.2	14.8	6.0	34.9 (± 98%)	
	24.2	37.7	17.1	9.2	22.1 (± 55%)	27.4.4 (± 5.79)
250 µg/L	91.8	147.0	50.3	64.9	88.5 (± 48%)	
	76.0	120.0	49.7	72.4	79.5 (± 37%)	
	43.7	53.2	41.9	43.1	45.5 (± 12%)	71.2.2 (± 7.43)
500 µg/L	132.1	150.0	113.0	171.2.1	141.6 (± 18%)	
	143.2	176.0	107.0	157.8	146.0 (± 20%)	
	139.1	174.5	638.7	186.1	284.6 (± 83%)	190.7.7 (± 28.01)
1000 µg/L	282.4	362.7	262.9	405.3	328.3 (± 20%)	
	27.48.3	354.8	27.46.5	441.2	337.7 (± 23%)	
	259.6	332.8	295.5	505.9	348.4 (± 31%)	338.1.1 (± 24.47)
ZinClear IM® 50CCT						
250 µg/L	2.6	BD	BD	BD	0.7 (± 200%)	
	3.1	BD	BD	BD	0.8 (± 200%)	
	0.8	BD	BD	BD	0.2 (± 200%)	0.5 (± 0.32)
500 µg/L	BD	BD	20.3	36.5	14.2 (± 124%)	
	0.8	BD	BD	3.4	1.1 (± 154%)	
	1.5	5.0	BD	3.2	2.4 (± 90%)	5.9 (± 2.06)
1000 µg/L	1.7	BD	17.2	7.2	6.5 (± 119%)	
	1.5	BD	BD	183.3	46.2 (± 198%)	
	1.8	BD	BD	169.5	42.8 (± 197%)	31.9 (± 17.67)

Coral Assessments

The ZnO treatment corals had various physical responses during the 48-h exposure. Representations of coral stress response are depicted in Figure 2. Corals in the control group had no physical condition changes throughout the whole exposure, with fully extended polyps and no mucus production (Figure 2A). Corals exposed to 27.4 µg/L ZnO exhibited mild polyp retraction

and slightly elevated mucus production after 12 h (Figure 2B). As the exposure continued, evident polyp retraction and closure persisted in addition to a slight lightening of coloration, thinning of coenenchyme, and increased mucus production (Figure 2C). Corals exposed to 71.2 µg/L ZnO exhibited mild polyp retraction, lightening of tissue coloration, and mucus production after 6 h of exposure (Figure 2D). After 24 h, mild to moderate changes in condition were observed, especially in polyp retraction and closure, lightening of coloration, thinning of the coenenchyme, and elevated mucus production (Figure 2E). Corals continued to decline, with severe polyp retraction, severe tissue thinning, and tissue loss by the end of the exposure (Figure 2F).

Corals exposed to 190.7 µg/L ZnO began to exhibit mild polyp retraction after 2 h that quickly progressed to severe retraction after 12 h (Figure 2D). Mild lightening of coloration was evident with multiple mucus strands produced. Corals in this treatment had severe responses after 48 h, including the appearance of skeletal ridges, partial tissue bleaching, and tightly retracted polyps (Figure 2F). Immediate responses were observed in the 338.1 µg/L ZnO treatment with moderate polyp retraction, slight mucus production, and apparent swelling of the coenenchyme after 2 h of exposure (Figure 2D). After 24 h, corals had severe reactions to the highest concentration of ZnO including significant lightening of color that led to bleached areas in the tissue and serve polyp retraction with exposed skeletal ridges (Figure 2F). Rapid declines in coral condition were observed after 48 h of exposure, with a high percentage of tissue mortality in the 338.1 µg/L ZnO treatments.

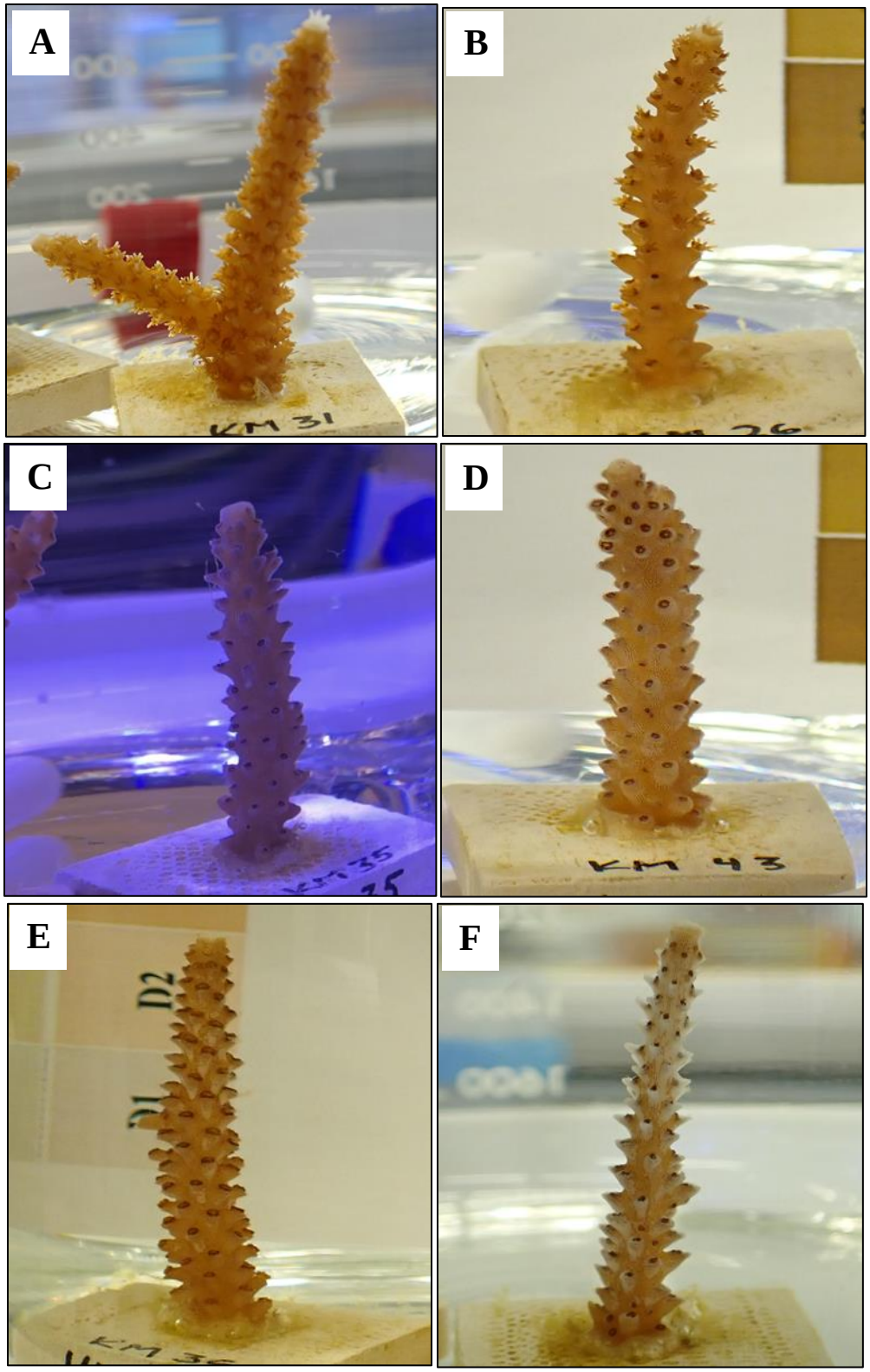


Figure 2. Variety of physical responses to the associated treatment during the exposure. A) Fully extended polyps, 24-h, control B) Partial polyp retraction, 6-h, 250 µg/L ZnO C) Slight elevation in mucus, 12-h, 500 µg/L ZnO D) Lightening in color and tissue swelling, 12-h, 500 µg/L ZnO E) Thinning of coenenchyme 48-h, 250 µg/L ZnO F) Severe tissue attenuation and mortality, 24-h, 500 µg/L ZnO.

The mean coral condition scores for the ZnO 48 h exposure are shown in Figure 3. Significant treatment effects were observed in the treatment groups at 2 h in the ZnO exposure (Kruskal-Wallis ANOVA, $p < 0.05$). Coral condition scores were significantly higher in the 190.7 µg/L ZnO and 338.1 µg/L ZnO treatment groups compared to the controls at 2 h after the start of the exposure (Multiple comparisons, $p = 0.003$ and $p = 0.0001$, respectively). The 71.2 µg/L ZnO, 190.7 µg/L ZnO, and 338.1 µg/L ZnO exposed corals scored significantly higher at 6 h ($p = 0.019$, $p = 0.011$, and $p = 0.0005$, respectively). All coral condition scores were significantly higher in all of the treatment groups compared to the controls after 12 h (Kruskal-Wallis ANOVA, $p < 0.05$).

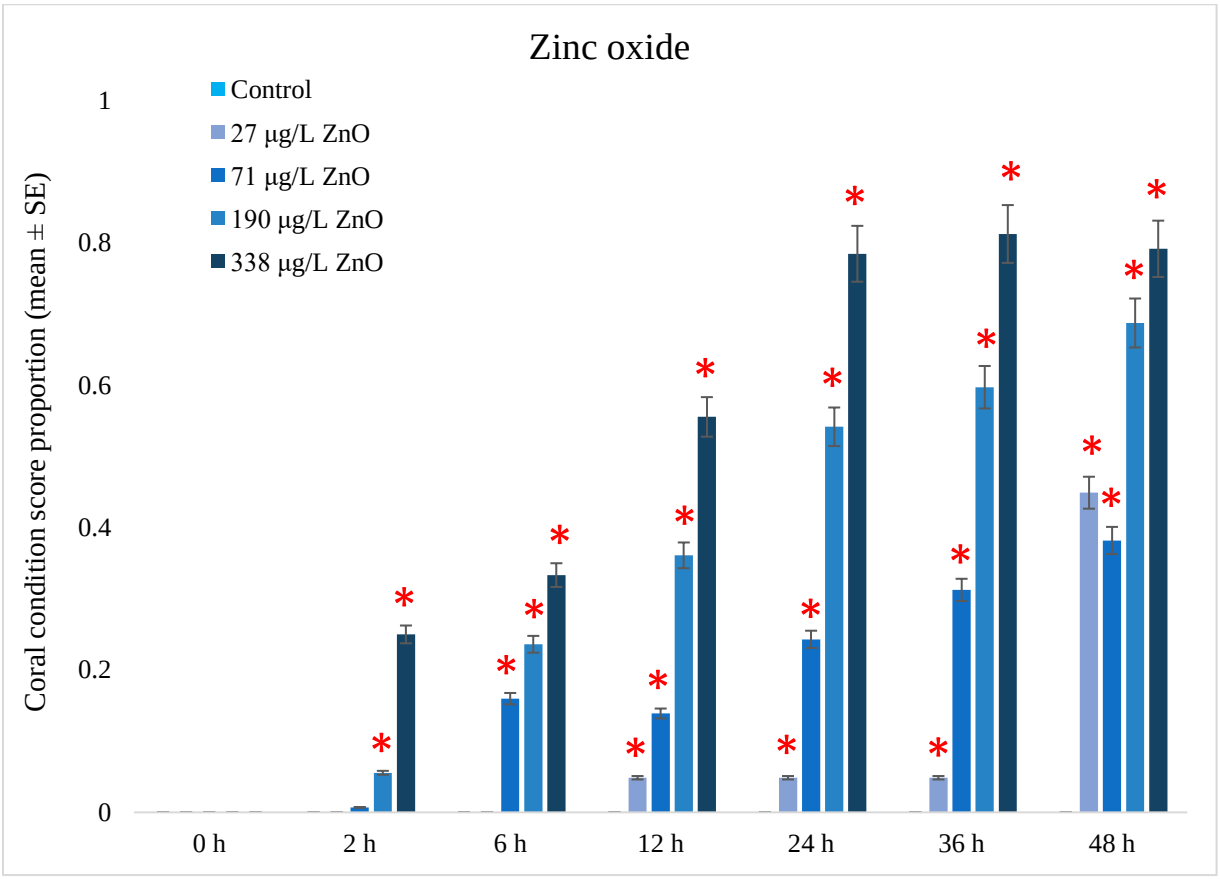


Figure 3. Mean coral condition score proportion (±SE) at each time point for the ZnO exposure based on the diagnostic criteria from Table 1. Red stars (*) denote significant differences ($p < 0.05$) between the treatment group and the control.

The mean coral condition scores for the ZinClear IM® 50CCT 48 h exposure are shown in Figure 4. Control corals and 1 µg/L ZinClear showed no changes in physical conditions with no mucus production during the 48 h exposure. Mild polyp retraction and mucus production were

detected after 24 h in the 6 µg/L ZinClear corals, and after 36 h in the 32 µg/L ZinClear corals, which persisted for the remainder of the exposure. After 48 h, only one treatment group, the 32 µg/L ZinClear treatment scored significantly higher compared to the control (Kruskal-Wallis ANOVA, p = 0.024).

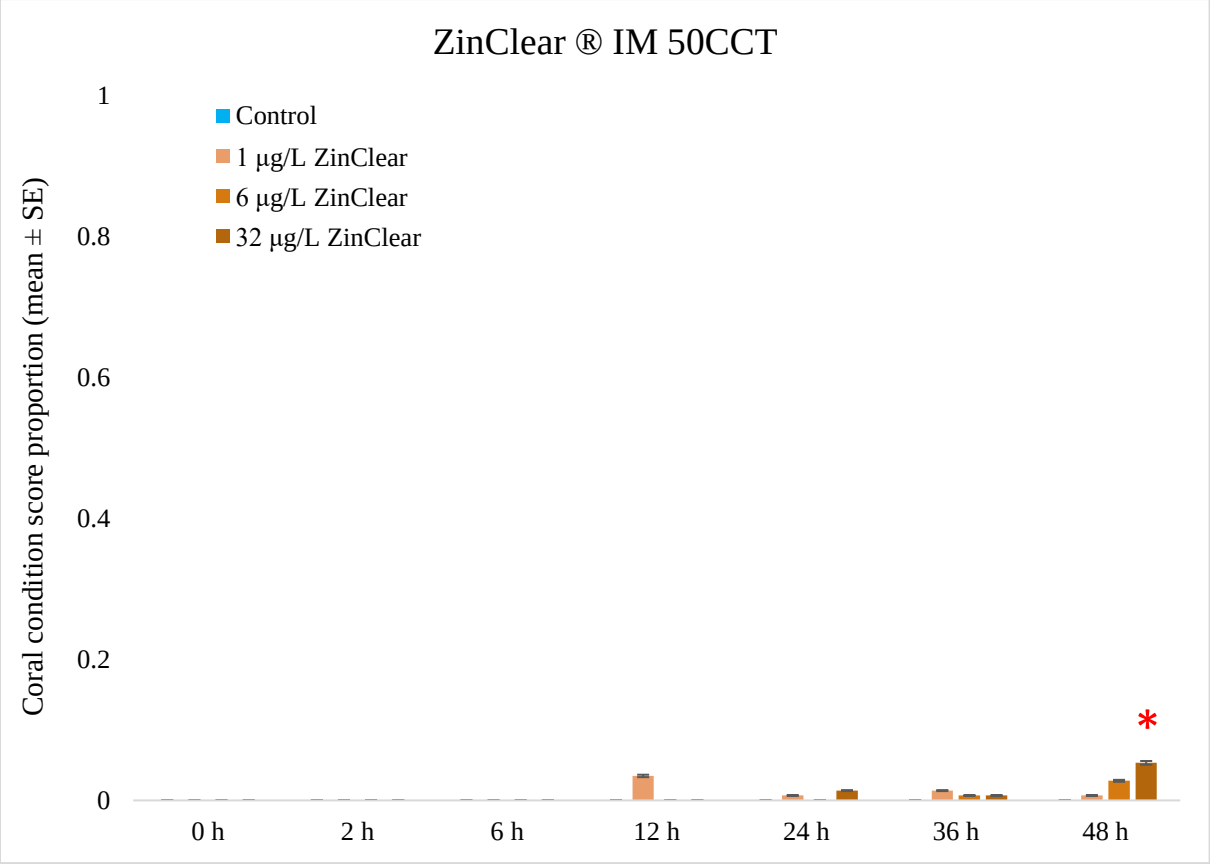


Figure 4. Mean coral condition score proportion (±SE) at each time point for the ZinClear IM® 50CCT exposure based on the diagnostic criteria from Table 1. Red stars (*) denote significant differences ($p < 0.05$) between the treatment group and the control.

Photosynthetic Efficiency

Dark-adapted maximum quantum yields (F_v/F_m) for the ZnO and the ZinClear IM® 50CCT exposures are shown in Figure 5. Photosynthetic efficiency measurements were taken pre- and post-exposure to measure the efficiency of the energy transfer between PSII and PSI. Significant effects were observed at the end of the exposure (Kruskal-Wallis ANOVA, $p < 0.05$), and the post-hoc analysis indicated that the corals in the 190.7 µg/L ZnO and 338.1 µg/L ZnO treatments had significantly decreased photosynthetic efficiency at 48 h compared to the control group (p = 0.041

and p = 0.028, respectively). The 27.4 µg/L ZnO, 71.2 µg/L ZnO, and all ZinClear IM® 50CCT treatment groups were not significantly different compared to the controls ($p > 0.05$).

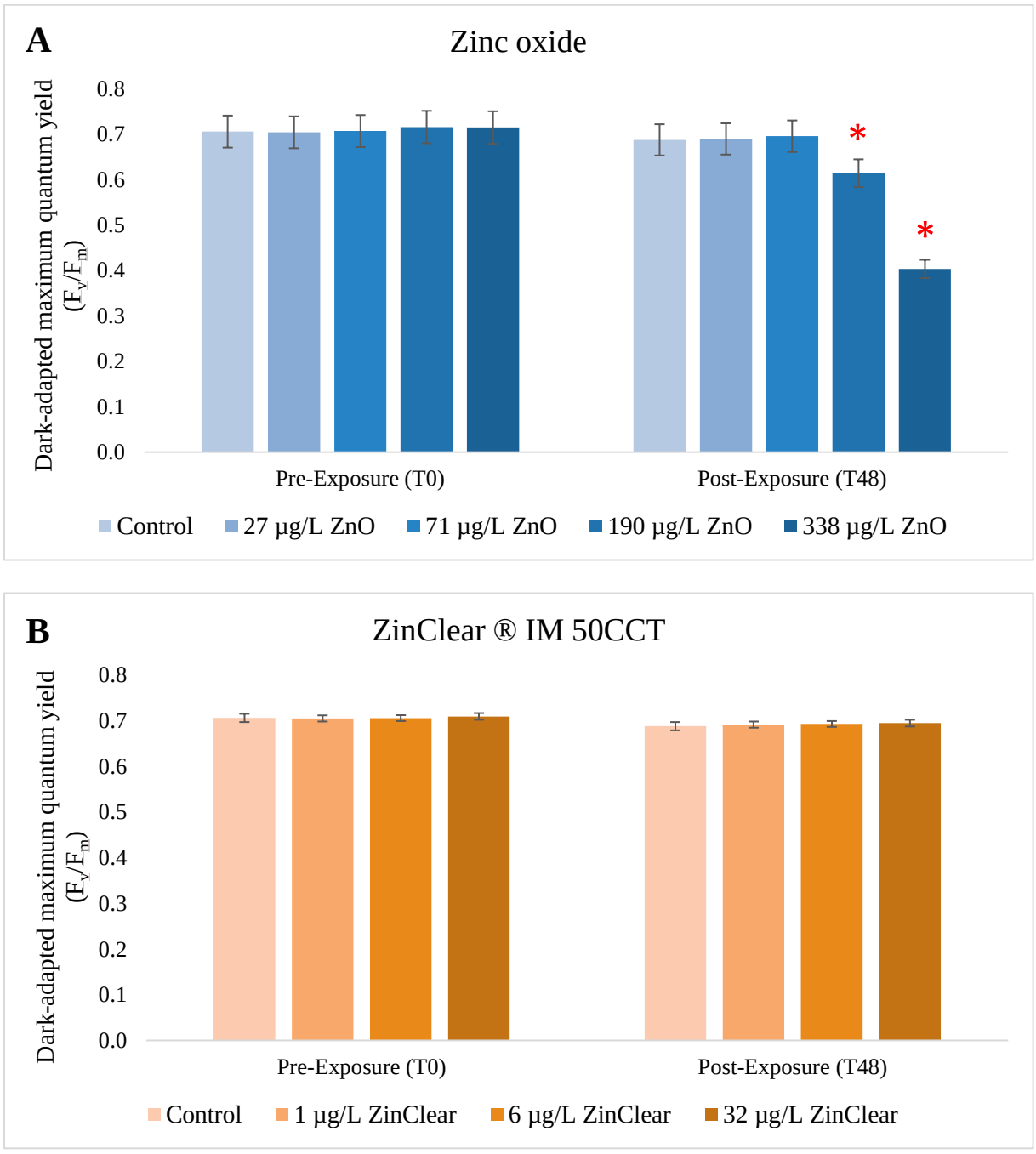


Figure 5. Dark-adapted maximum quantum yield (±SE) for each treatment of the ZnO and the ZinClear IM® 50CCT group at 0 h and 48 h. Red stars (*) denote significant differences (p < 0.05) between the treatment group and the control.

Calcification

The mean normalized skeletal growth rates for the ZnO and ZinClear IM® 50CCT exposures, expressed as percent change per day, are shown in Figure 6. No significant differences as a result of the exposures were observed (Kruskal-Wallis ANOVA, p = 0.176).

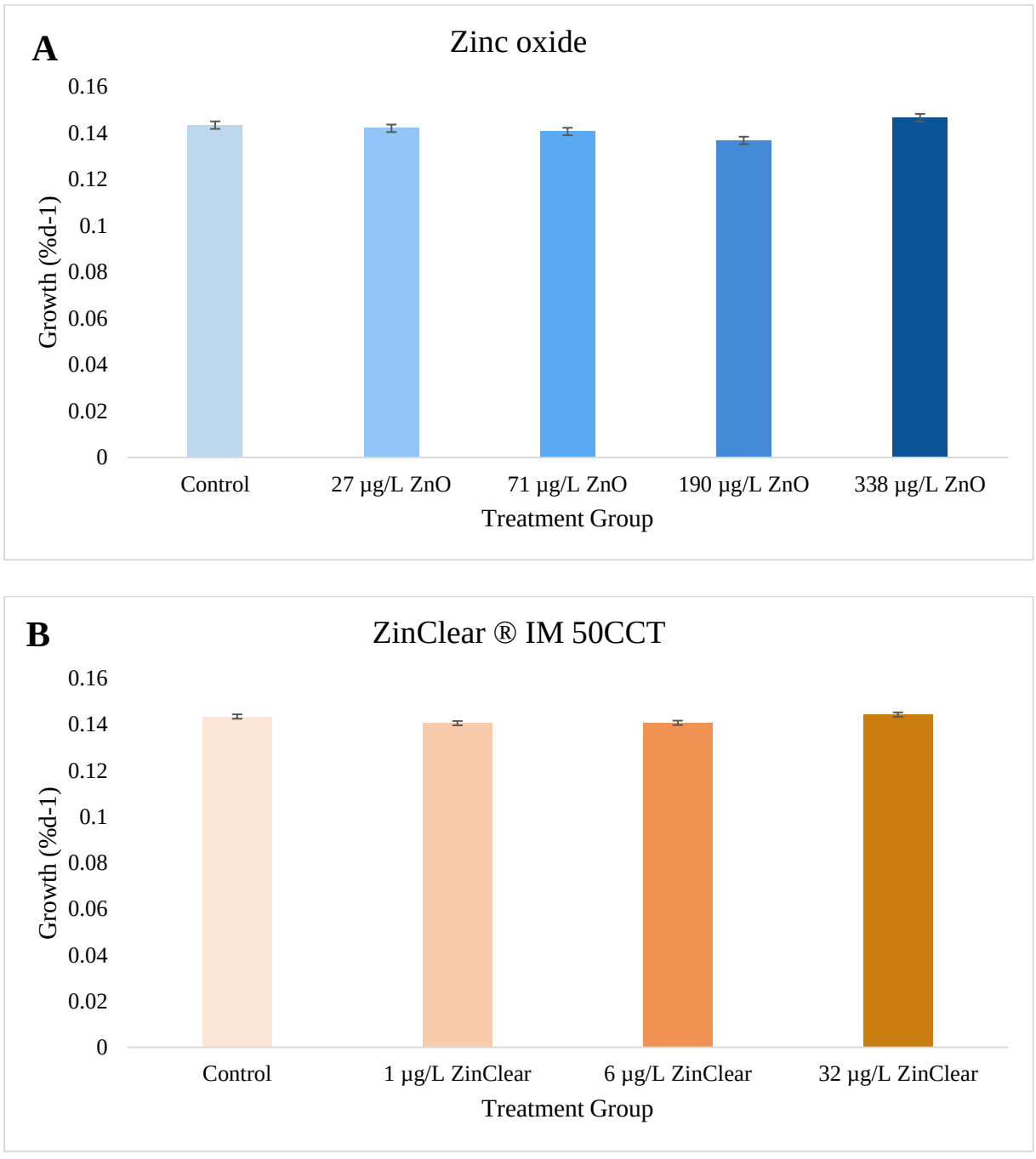


Figure 6. Mean normalized growth rate (±SE) for each treatment in the ZnO and the ZinClear IM® 50CCT group.

Mortality

Figure 7 shows the mean percent mortality at each time point. After the 48 h, the 71.2 µg/L, 190.7 µg/L, 338.1 µg/L ZnO treatment groups had 21.7%, 65.4%, and 96.6% mean mortality, respectively. Multiple corals had 100% tissue mortality at the end of the exposure in both the 190.7 µg/L ZnO and the ZnO treatment groups. No mortality was observed in the control corals, the 27.4 µg/L ZnO, or corals in any of the ZinClear IM® 50CCT treatments after 48 h.

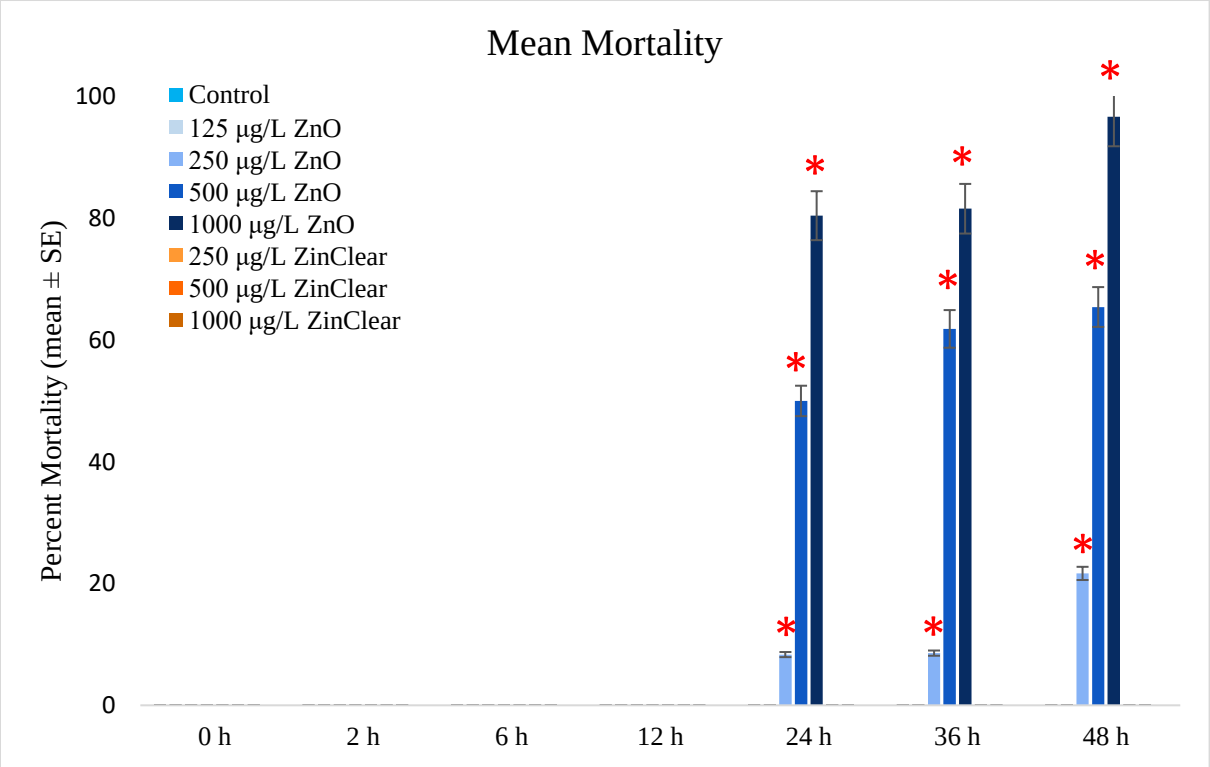


Figure 7. Mean percent mortality (±SE) for each treatment in the ZnO and the ZinClear IM® 50CCT group. Red stars (*) denote significant differences ($p < 0.05$) between the treatment group and the control.

Endpoints

Lethal (mortality) and sublethal (coral condition) dose-response curves for the ZnO exposure at 24, 36, and 48 h are provided in Figure 8. The 24-h EC_{50} was 152.9 µg/L ZnO (Figure 8A), the 36-h EC_{50} was 131.1 µg/L ZnO (Figure 8C), and the 48-h EC_{50} was 101.5 µg/L ZnO (Figure 8E). Similarly, the 24-h LC_{50} was 156.6 µg/L ZnO (Figure 8B), the 36-h LC_{50} was 148.1 µg/L ZnO (Figure 8D), and the 48-h LC_{50} was 117.0 µg/L ZnO (Figure 8F).

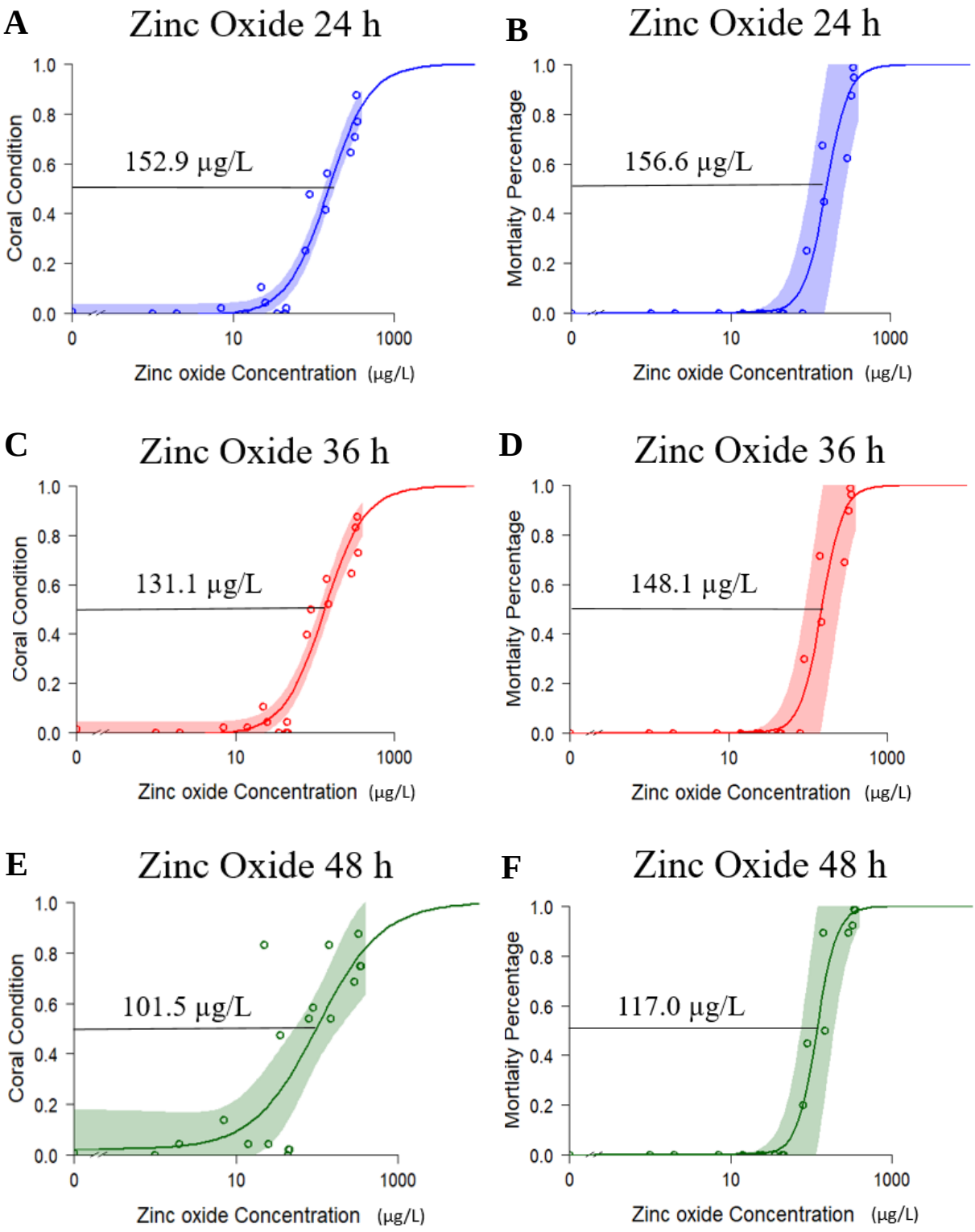


Figure 8. Sublethal and lethal dose-response curves and estimated thresholds for the effects of 24, 36, and 48 h exposure to ZnO in *Acropora cervicornis*. A) Coral condition at 24 h, B) Mortality at 24 h, C) Coral condition at 36 h, D) Mortality at 36 h, E) Coral condition at 48 h, F) Mortality at 48 h.

Titanium Dioxide Exposure

Water Quality

A summary of the water quality data is provided in Table 4. No significant differences were found (Kruskal-Wallis ANOVA, $p > 0.05$) in water quality parameters at the start of the exposure between the control and treatment groups. Post exposure, PO_4 was significantly greater in the 10 mg/L TiO_2 treatment groups compared to the control (Multiple comparison, $p = 0.0004$). There were no significant differences between temperature, pH, alkalinity, NO_2, NO_3, and NH_3 in any of the TiO_2 and UV Cut treatments compared to the controls ($p > 0.05$). The only parameter not tested was dissolved oxygen due to a mechanical error of the dissolved oxygen probe.

Table 4. Mean (± SD) water quality parameters at 48 h for TiO_2 and UV Cut exposures.

Treatment	Temp (°C)	pH	Alk (ppm)	PO_4 (ppm)	NH_3 (ppm)	NO_2 (ppm)	NO_3 (ppm)
Control	26.6 (±0.66)	8.22 (±0.01)	117 (±4.34)	0.11 (±0.04)	0.00 (±0.00)	0.01 (±0.00)	0.01 (±0.00)
Titanium Dioxide							
1.25 mg/L	26.0 (±0.61)	8.20 (±0.01)	115 (±4.00)	0.11 (±0.01)	0.00 (±0.00)	0.01 (±0.00)	0.01 (±0.00)
2.5 mg/L	26.8 (±0.23)	8.19 (±0.00)	112 (±1.05)	0.11 (±0.05)	0.00 (±0.00)	0.01 (±0.00)	0.01 (±0.00)
5.0 mg/L	26.2 (±0.36)	8.22 (±0.01)	120 (±1.12)	0.12 (±0.03)	0.00 (±0.00)	0.01 (±0.00)	0.01 (±0.00)
10.0 mg/L	26.4 (±0.55)	8.21 (±0.02)	118 (±4.42)	0.07 (±0.01)	0.00 (±0.00)	0.01 (±0.00)	0.01 (±0.00)
UV Cut							
2.5 mg/L	26.6 (±0.25)	8.18 (±0.01)	117 (±4.08)	0.09 (±0.03)	0.00 (±0.00)	0.01 (±0.00)	0.01 (±0.00)
5.0 mg/L	25.9 (±0.06)	8.18 (±0.01)	120 (±3.19)	0.13 (±0.05)	0.00 (±0.00)	0.01 (±0.00)	0.01 (±0.00)
10.0 mg/L	26.6 (±0.55)	8.18 (±0.01)	118 (±1.56)	0.11 (±0.02)	0.00 (±0.00)	0.01 (±0.01)	0.01 (±0.00)

Exposure Concentrations

Nominal concentrations were used to determine threshold values for the titanium dioxide exposure, as the methodology for this compound has not yet been finalized with the laboratory. Samples have been preserved until analysis can be completed.

Coral Assessments

Throughout the 48 h TiO_2 and UV Cut exposures, treated corals showed mild to no response (Figure 9). Control corals maintained full polyp extension with no mucus production and no other changes in physical condition (Figure 9A). Corals exposed to 1.25 mg/L TiO_2 had mild mucus production at the start of the exposure, but appeared to return to normal behavior as the exposure continued. At 24 h, corals exposed to 1.25 mg/L TiO_2 had mild to moderate effects present including evidence of polyp retraction and slight closure of the oral disc (Figure 9C). Similar behavior was observed in the corals exposed to 2.5 mg/L TiO_2 with slight mucus production and mild polyp retraction (Figure 9D).

Corals exposed to 5 mg/L TiO_2 exhibited minor tissue swelling and mild polyp retraction after 36 h that subsided by the end of the exposure (Figure 9E). Corals exposed to 10 mg/L TiO_2 exhibited mild polyp retraction after 24 h that progressed to moderate polyp retraction and partial polyp closure after 48 h (Figure 9F). However, this was only evident in one replicate of this treatment. Due to the lack of response, no significant differences in mean coral condition score were observed as a result of the TiO_2 exposure after 48 h (Kruskal-Wallis ANOVA, $p > 0.05$) (Figure 10).

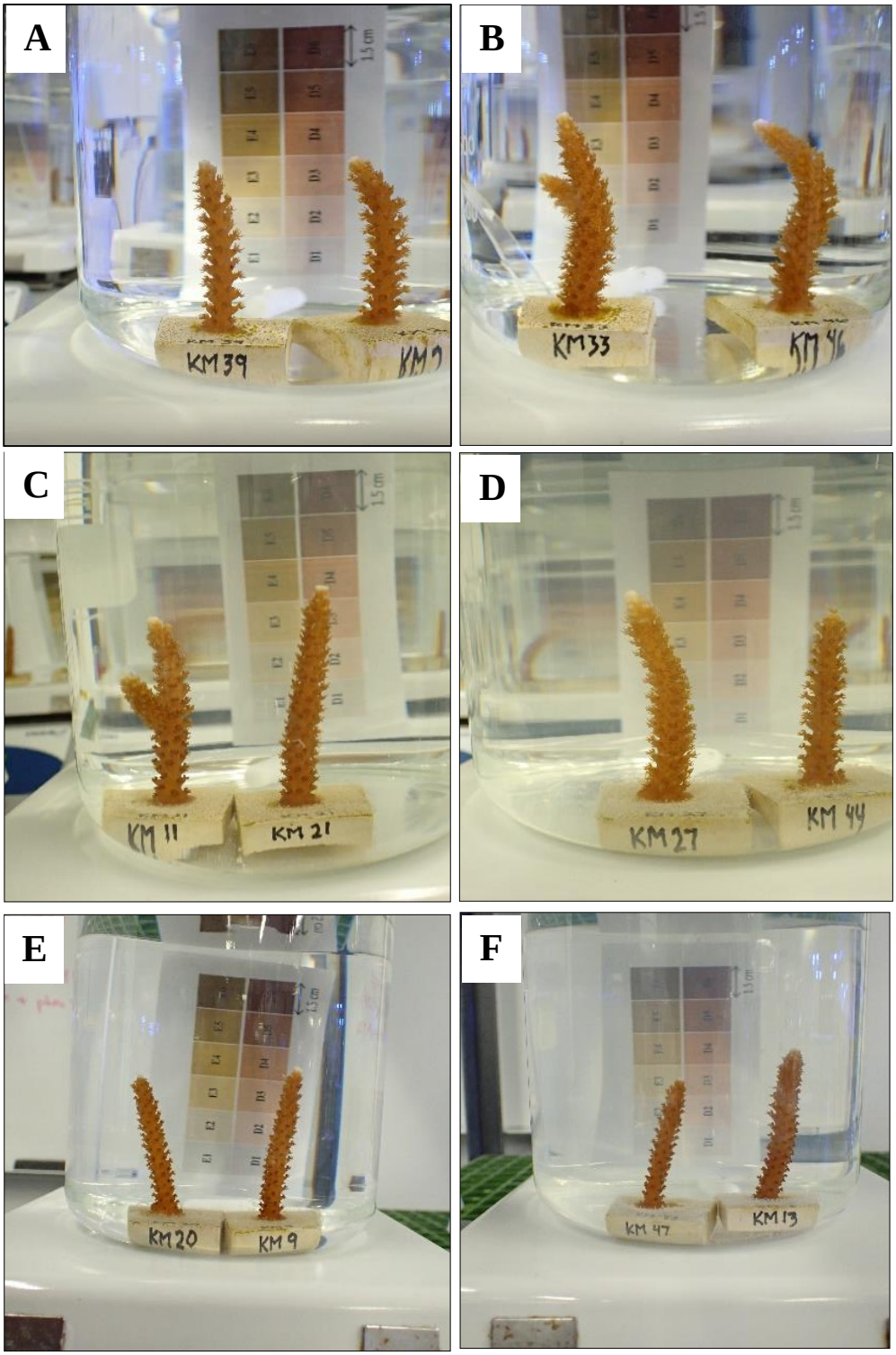


Figure 9. The physical responses to the associated treatments during the 48-h exposure. A) Fully extended polyps, 2-h, control, B) Fully extended polyps, 6-h, 5 mg/L UV Cut, C) Slight polyp retraction, 12-h, 2.5 mg/L TiO_2, D) Mild polyp retraction, 36-h, 5 mg/L TiO_2, E) Mild tissue swelling, 48-h, 1.25 mg/L TiO_2, F) Moderate polyp retraction with partial polyp closure, 48-h, 10 mg/L TiO_2.

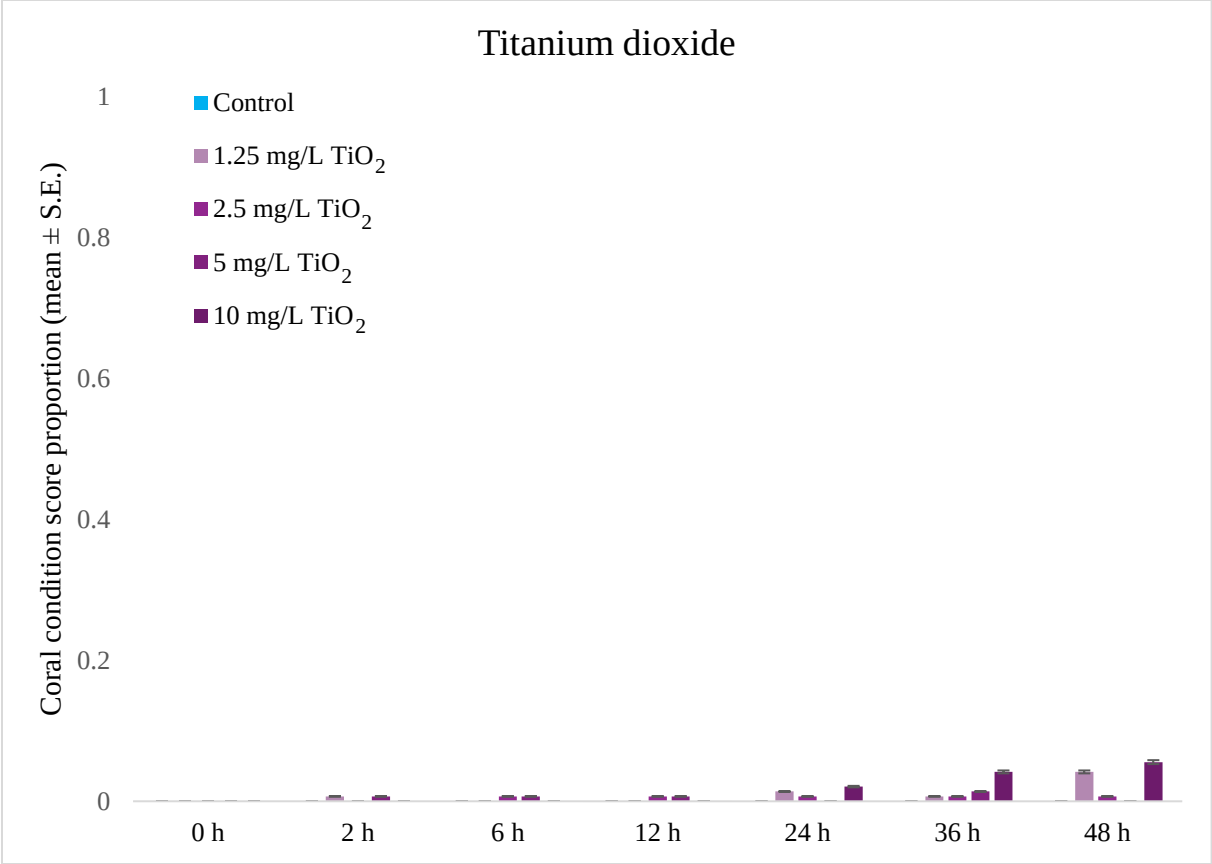


Figure 10. Mean coral condition score proportion (±SE) at each time point for the TiO_2 exposure based on the diagnostic criteria from Table 1.

Corals exposed to 2.5 mg/L and 5 mg/L UV Cut had no change in physical condition at any point during the 48 h exposure (Figure 9B). Similar to the controls, these corals had normal polyp extension and mucus production. Corals in the 10 mg/L UV Cut treatment had minor swelling of the coenenchyme at 12 h and slight tissue swelling and polyp retraction at 36 h, which resolved by the end of the exposure. No significant differences in mean coral condition scores were observed as a result of exposure to UV Cut after 48 h (Kruskal-Wallis ANOVA, $p > 0.05$) (Figure 11).

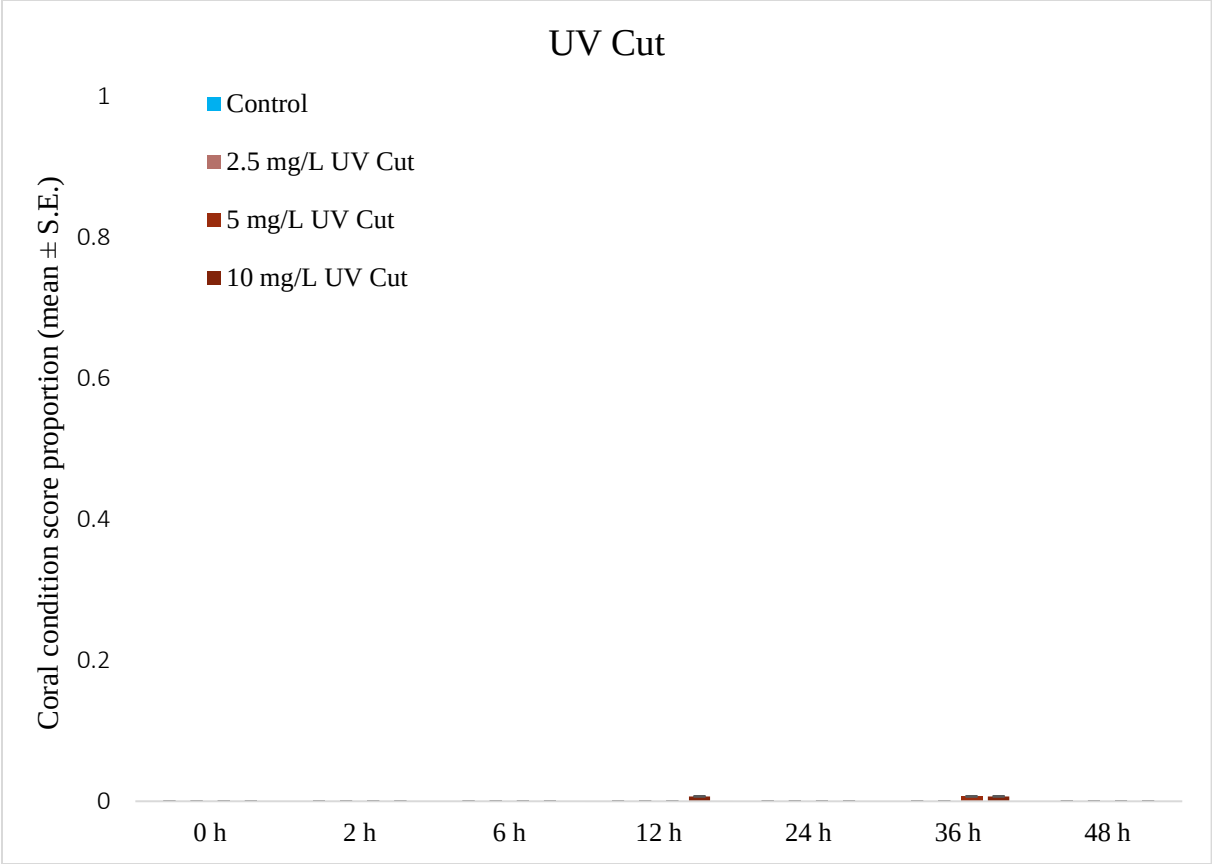
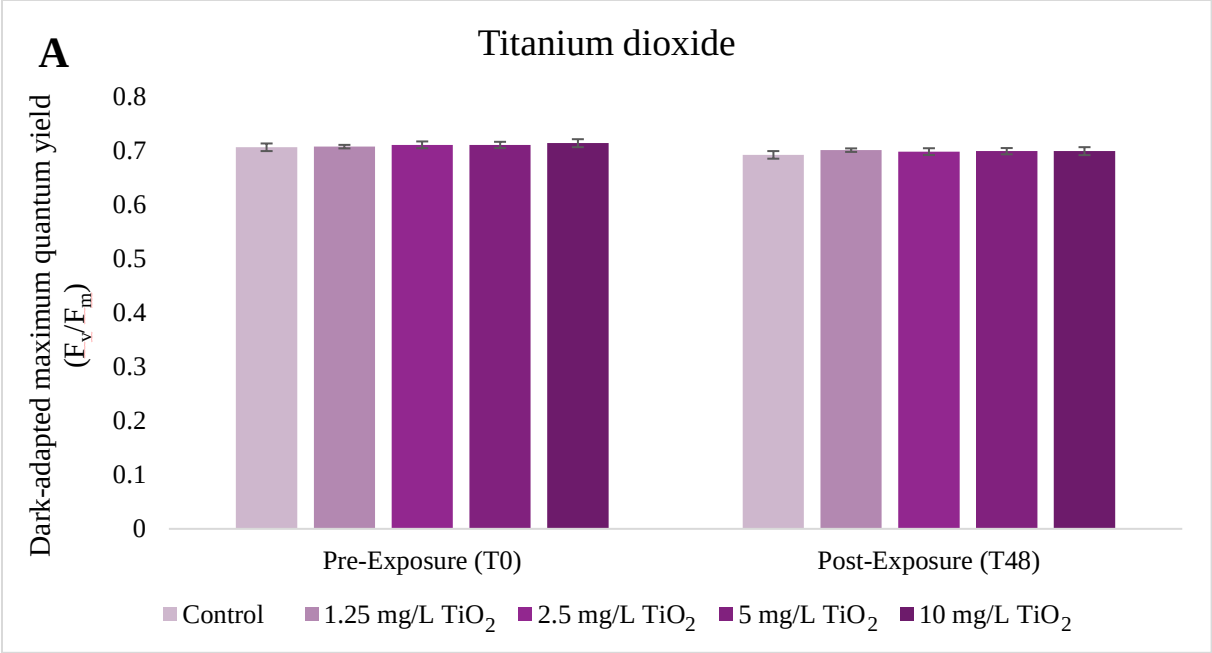


Figure 11. Mean coral condition score proportion (±SE) at each time point for the UV Cut exposure based on the diagnostic criteria from Table 1.

Photosynthetic Efficiency and Calcification

The dark-adapted maximum quantum yield (F_v/F_m) for the TiO_2 and UV Cut (pre-exposure and post-exposure) is shown in Figure 12. No significant differences between treatments were found (Kruskal-Wallis ANOVA, $p > 0.05$), although a few of the post-exposure measurements showed a slight, non-significant increases in mean maximum quantum yield.



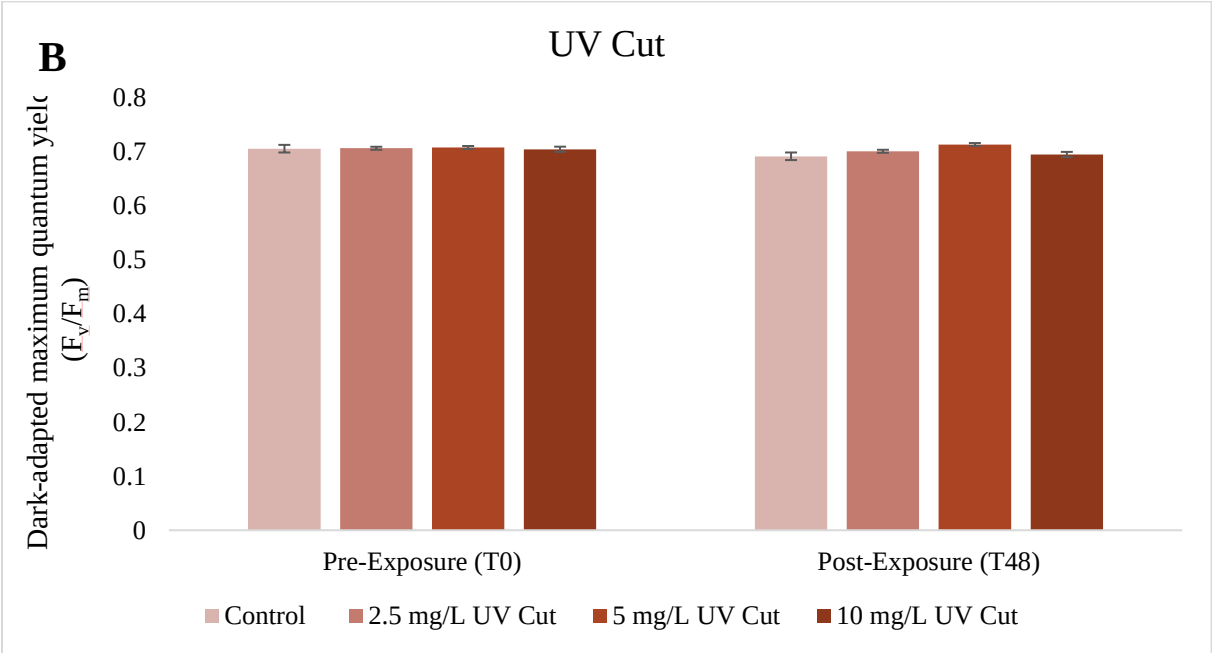


Figure 12. Dark-adapted maximum quantum yield (±SE) for each treatment of the TiO_2 and UV Cut group at 0 h and 48 h.

The mean normalized growth rates (expressed as percent change per day) for the TiO_2 and UV Cut exposure is shown in Figure 13. No significant differences in post-exposure growth rate were found (Kruskal-Wallis ANOVA, $p > 0.05$).

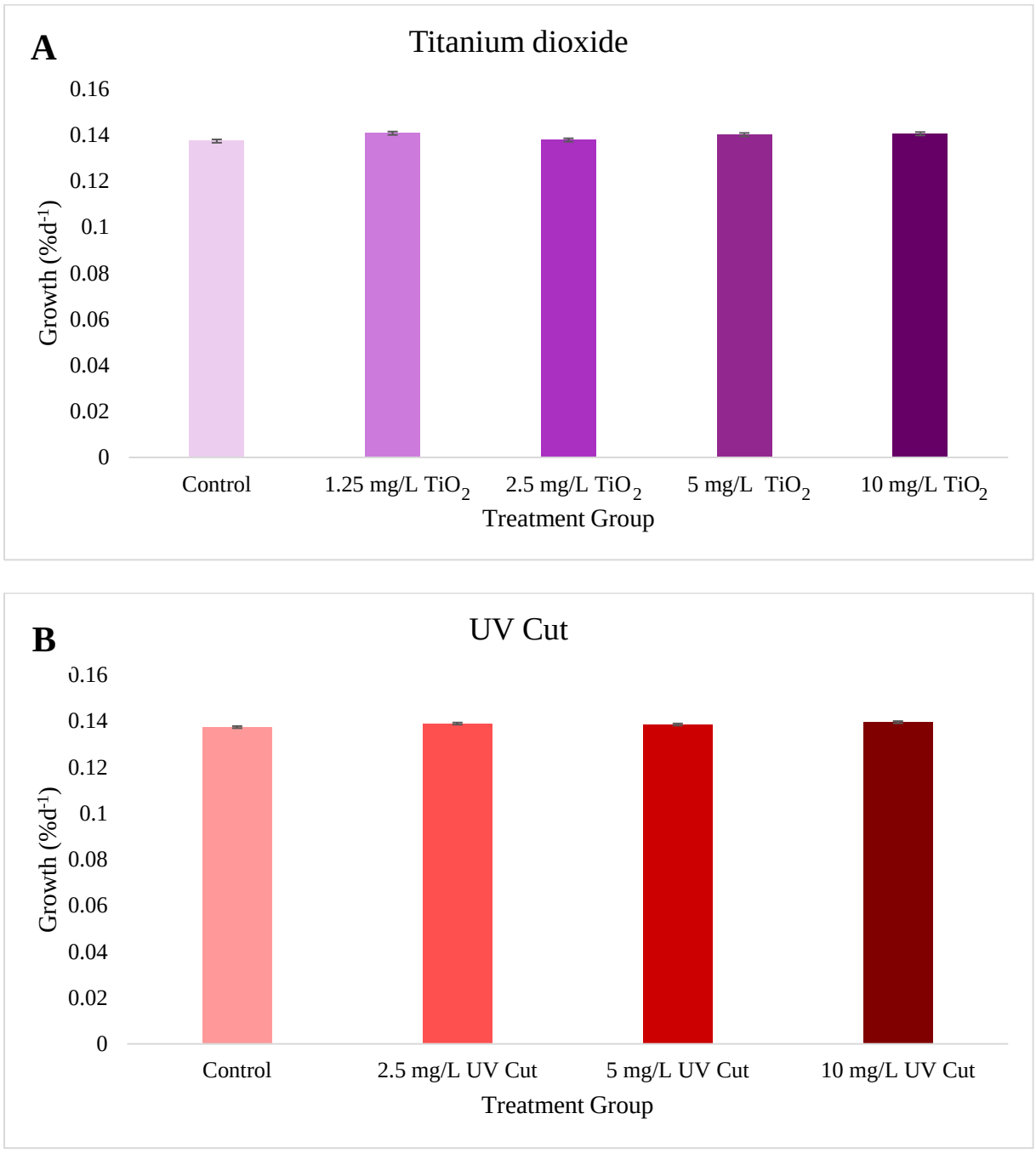


Figure 13. Mean normalized growth rate (±SE) for each treatment in the TiO_2 and UV Cut group.

Endpoints and Mortality

As no mortality or significant changes in coral condition occurred during the 48 h exposures, sublethal (EC_{50}) and lethal (LC_{50}) thresholds for TiO_2 and UV Cut could not be determined.

DISCUSSION

The purpose of this study was to determine and compare the acute toxicological impacts of the sunscreen ingredients zinc oxide, titanium dioxide, and two commercial sunscreen formulations to *A. cervicornis*. *Acropora cervicornis* exhibited stronger responses to the unmodified forms (ZnO and TiO_2) compared to the modified products (ZinClear IM® 50CCT and UV Cut). Corals exposed to 190.7 µg/L ZnO and 338.1 µg/L ZnO had immediate and severe responses that lead to significant mortality at the end of the 48 h exposure. In contrast, coral exposed to TiO_2 exhibited mild to moderate responses that resolved by the end of the exposure. Additionally, ZinClear and UV Cut exposed corals displayed normal behavior with insignificant reactions during the exposure. No mortality occurred in corals as a result of exposure to TiO_2, ZinClear, or UV Cut, limited changes in coral condition and lack of mortality in these exposures provided inadequate data to estimate sublethal and lethal thresholds.

Declines in the mean maximum quantum yield in the two highest concentrations of ZnO after 48 h exposure can be attributed to loss of zooxanthellae and coral mortality. This is similar to previous studies where both declines in photosynthetic efficiency and zooxanthellae damage were observed (Corinaldesi et al. 2018, Fel et al. 2019). Fel et al. (2019) found decreases in the maximal photosynthetic efficiency in a chronic (35 d) exposure to *Stylophora pistillata*, at the highest concentration of 860 µg/L ZnO. Almost an immediate decline in the dark-adapted mean maximum quantum yield and total tissue bleaching was observed after 15 d; however, the dark-adapted methodology in Fel et al. (2019) only maintained corals in the darkness for 10 minutes before measurements were taken, which may not have been sufficient for accurate readings (Ralph et al. 2016).

In an acute exposure (48 h) to 6.3 mg/L ZnO (uncoated particles of size ranging from 20 to 200 nm) in *Acropora spp.*, Corinaldesi et al. (2018) observed a significant amount of zooxanthellae release and damaged cells that were characterized as pale and transparent. Corals

were observed to be partially bleached after 24 h, which progressed to almost 70% bleaching at the end of the exposure. Despite the higher concentration of zinc oxide tested in Corinaldesi et al. (2018), fewer effects were observed in the present study, where declines in photosynthetic efficiency occurred in corals exposed to ZnO concentrations as low as 190.7 µg/L. This could be explained due to the larger size of the nanoparticles used by Corinaldesi et al. (2018) with some of the particles ranging up to 200 nm in size. Additionally, bleaching in Corinaldesi et al. (2018) was based on a scale organized in ranks (including 0-10% tissue mortality as no presence of mortality and 60% mortality categorized as total bleaching), and the scoring for exposed corals was based multiple photographs in taken at the beginning of the exposure in comparison to the end of the 48 h exposure, which might not be as accurate or quantitative as the diagnostic criteria found in Table 1 above.

Corinaldesi et al. (2018) also compared two different modified forms of TiO_2; one was coated with alumina and dimethicone, while the other had manganese incorporated into the lattice structure of the compound. These particular modifications are one of the techniques used to prevent free radicals and reduce the impact to marine organisms (Corinaldesi et al. 2018). Exposure to these two compounds resulted in minimal alterations in the cell structure of the zooxanthellae and less than 10% of bleaching was present throughout the acute exposure (48 h) to 6.3 mg/L ZnO. Although Corinaldesi et al. (2018) did not use an unmodified form of TiO_2 for comparison under the same parameters, the concentrations of the TiO_2 forms were comparable to the nominal concentrations dosed in the present study, which resulted in a similar lack of effects on coral condition. Thus exposure to TiO_2 is does not appear to have impacts to coral, and when TiO_2 is coated with other compounds, it has even less of an effect (Corinaldesi et al. 2018).

Coating mineral UV filters with other compounds such as alumina or dimethicone could minimize the reactivity in seawater compared to uncoated nanoparticle forms. Fournier et al. (2021) observed a reduction in the production of free radicals over a 4 h period when coated nanoparticles were exposed to UV radiation, in particular lignin-protected TiO_2. Nanoparticles with surface coatings of aluminum oxide (Al_2O_3) had a lower percentage of degradation and lower toxicity in marine and freshwater environments over a 48-h exposure (Slomberg et al. 2021). However, Slomberg et al. (2021) documented that silicon dioxide (SiO_2) coated nanoparticles dissolved quickly in all exposed aqueous solutions that could become phototoxic and potentially

bioavailable in the water column. The long-term impacts of uncoated particles in comparison to coated particles and their role in marine ecosystems is largely unknown and thus cannot be considered a coral-safe alternative until further research is conducted.

Continuing studies on mineral UV filters are needed to determine the chronic effects of sunscreen products on coral species, nanoparticles and coated modified forms of these chemicals. This will further bridge the knowledge gap on how these compounds may accumulate in marine environments and the potential for the production of ROS species with newly modified coated ingredients. Investigations into genetic and cellular effects in coral resulting from exposure to these compounds would assist in evaluating the relative severity of potential environmental impacts. The additional information would be particularly useful for examining the molecular level effects of TiO_2 exposure in corals, as declines in corals condition and mortality were not observed in this study.

CONCLUSION

The findings from this research demonstrate that expose to zinc oxide has the potential to cause acute rapid deterioration and mortality in *A. cervicornis* at relatively low concentrations. Despite the limited effects caused by acute exposure to the commercial product ZinClear, prolonged exposure could have the potential to lead to a decline in photosynthetic efficiency, bleaching, and mortality. With the additional knowledge gained from these results, the safety of ZnO in sunscreen products requires further investigations and products with this chemical should not be listed as coral-safe. In contrast, TiO_2 was observed to have mild effects on coral condition and no mortality. Although TiO_2 and the two commercial products did not have a significant effect on coral condition, extensive standardized testing is required before any reef-safe labeling may be considered. Future studies should also include chronic exposures at concentrations typical of marine environments. Application of results from this study and future research using quantitative methodologies can support environmentally conscious decision making and evaluation current and future sunscreen formulations.

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