4-27-2018

Toxicological Effects of Commercial Sunscreens on Coral Reef Ecosystems: New Protocols for Coral Restoration

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Capstone of
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Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science

M.S. Marine Biology

Nova Southeastern University
Halmos College of Natural Sciences and Oceanography

April 2018

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HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

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By

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April 2018

Abstract

The primary purpose of consumer-grade sunscreen is to protect skin from harmful UVA and UVB rays. This market has grown during the past 80 years, and environmental contamination from increasing amounts of sunscreen compounds have created concern. In particular, impacts on ocean ecosystems have inspired investigations and toxicological research on their effects on marine life. Unfortunately, such studies using marine flora and fauna are scarce, and the impact of chemical exposure to consumer sunscreens is neither adequately measured nor completely understood. In a pilot study by the Coral Restoration Foundation, *in situ* toxicity exposure to 10 different brands of sunscreens was performed on the Caribbean scleractinian staghorn coral, *Acropora cervicornis*. Coral samples were ranked on tissue degradation following the sunscreen exposure, however no significant differences were found between exposed and control samples. Additional studies should be performed to better understand other possible sub-lethal effects. One such application is in the proper handling of corals during restoration; as other compelling evidence indicates, sunscreens have the potential to be toxic depending on concentration and exposure time, among other factors. This literature review revealed that sunscreens containing only non-nano zinc oxide or non-nano titanium dioxide as primary UV filters may best reduce stress to marine organisms and coral fragments in coral nurseries.

Keywords: UV filter, toxicity, Acropora cervicornis, marine toxicology, chemical pollution, contaminant, pollutant

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1. Introduction

1.1 Statement of Purpose

Determining toxicity thresholds for particular compounds in diverse organisms presents many challenges. General toxicity studies of freshwater organisms are plentiful, but the complexity of seawater makes toxicity studies of marine organisms more involved (Baker *et al*., 2014). Existing methods for determining toxicity thresholds are insufficient due to variability in external parameters (such as light levels and salinity) and inconsistent methodologies. Additionally, it is argued whether these types of studies sufficiently model current environmental conditions (Chapman, 2007).

Only a few studies on UV filter toxicity include coral species (Danovaro *et al.*, 2008; Skelly *et al.*, 2012; Downs *et al*., 2014; Jovanović and Guzmán., 2014; Sharp *et al*., 2015; Downs *et al*., 2016; McCoshum *et al.*, 2016). Although other marine organisms are affected by UV filter toxicity, reef corals form the structural framework of the most biodiverse marine ecosystem. Thus, additional studies on sunscreen toxicity in corals will provide important data to help preserve our reefs. After sufficient toxicity data is collected, it is recommended that good management practices and government regulations would need to be implemented to control the release of sunscreens into the ocean, but this is beyond the scope of this paper. Presently, we are unaware of how various UV filters may affect scleractinian corals and marine ecosystems at large.

The purpose of this capstone project was to: (a) research the available data on UV filter toxicity to marine organisms, (predominantly corals) and how the data were obtained (traditional versus modern methodologies); (b) discuss, using principles of aquatic toxicology, UV filter toxicity to individual marine organisms versus ecotoxicology; (c) observe, at a histopathological level, the effects of various sunscreen filters *in situ* on the scleractinian coral *Acropora cervicornis*; and (d) use the results of the literature review and case study to recommend improvements for universal practices and standards when manipulating corals for conservation and research purposes.

1.2 History of Sunscreen

The use of topical UV filters to protect human skin from the sun's radiation dates as far back as ancient Egypt, evolving over the last century by manufacturers for the benefit of human health. The first documented use of a sunscreen occurred in the United States in 1928, made with the organic compounds benzyl cinnamate and benzyl salicylate as an emulsion (Wang and Hu, 2012). A similar composition was introduced in the 1930s by H.A. Milton Blake, an Australian chemist, who used phenyl salicylate (salol) (Rigel, 2004). Later, UV-filtering lotions appeared again in the United States but with quinine oleate and quinine bisulfate as the active ingredients (Lowe, 2006). By 1936, the demand for sun protectant increased, and cosmetic companies grew in revenue by manufacturing a new personal care product (PCP): sunscreen (Rebut, 1990). L'Oréal first coined the term "commercial sunscreen" in 1936, marketing the cosmetic agent as available to all consumers (Rigel, 2004).

During World War II, red veterinary petrolatum was issued to soldiers by the military for sun protection, although its protective effects were minimal, acting as a weak physical barrier against the sun (Rigel, 2004). In the 1940s, dermatologists began prescribing cream that contained p-aminobenzoic acid (PABA) as a UV filter which created opportunities for cosmetologists to develop new derivatives (Sulzberger *et al.*, 1947). Due to numerous allergy reports to PABA during the next several years, PABA was eventually removed from most cosmetic lotions, with the "PABA free" label gaining popularity in 1970 (Rigel, 2004). Benzophenone became the first compound in sunscreen to block UVA rays during the 1960s (Urbach, 2001), yet the regulation on its effectiveness from UVA exposure was poorly managed (Wang and Hu, 2012). Many sunscreen products made false or inadequate claims over UVA/UVB broad-spectrum protection well through the 1990s (Wang and Hu, 2012).

In 1977, Johnson&Johnson formulated the first "waterproof" sunscreen (Coppertone), and sunscreens were determined to be a "safe product" by the Food and Drug Administration (FDA) for consumer use; the sun protection factor (SPF) was also established as a method for consumers to know how well the product protected skin from solar irradiation (Sikes, 1998). Various sunscreens were considered "tanning oils" or "tanning lotions", with very low SPFs that offered protection against sunburn but not

general sun exposure (Wang and Hu, 2012). In 2012, the FDA affirmed that sunscreens containing an SPF of 15 or greater could aid in the reduction of skin cancer. However, poorly-defined labeling regulations failed to remove or enforce the identification of sunscreens below SPF 15 as non-protective (Wang *et al*., 2011). As a result, Americans today still purchase "sunscreens" that may reduce the likelihood of sunburn, but ultimately fail to resist UVA/UVB absorption by the skin; there is no current evidence that sunscreens below SPF 15 protect against cancer (Sharfstein, 2015). Not only is it still unclear how well sunscreen chemicals protect human health, but their toxic effects in the natural environment are also becoming a concern.

1.3 Economics and Marketing

Marine and coastal tourism continues to increase globally and is expected to attract about 1.56 billion tourists world-wide by 2020 (Honey and Krantz, 2007); the demand for sun care products is expected to also rise. Although a consumer-heavy country, the United States' sun care market represents 3% in retail value of the entire PCP market (Osterwalder, 2014), reporting $1.74 million in revenue in 2015 alone and expressing a mean annual growth rate of 35% between 2011 to 2015 (Research and Markets, 2016). As skin cancer awareness heightens and coastal tourism continues to steadily increase, the sunscreen market is projected to surpass its current growth pace, with worldwide sales increasing around 7% every year (Osterwalder, 2014). By recommendation of the FDA, an average of 20 g of lotion per application is considered adequate for sun protection (Poiger *et al.*, 2004), although it has been proposed that consumers may often apply substantially more than 20 g at one time (Giokas *et al.*, 2007). Consequently, the sun care industry responds to consumer demand for more product, while impacts of these chemical products on the environment are often overlooked or simply ignored.

1.4 Major Constituents

Sunscreens are "any cosmetic product containing UV filters in its formulation in order to protect the skin from solar deleterious UV-light" (Salvador and Chisvert, 2005). Therefore, UV filters are the major constituents in sunscreen products (and are the chemicals most often scrutinized). UV filters are grouped into two categories: organic

(chemical, e.g., benzophenones, cinnamates, camphor derivatives) and inorganic (mineral, e.g., titanium dioxide and zinc oxide and their nanoparticles [NP]). Both inorganic and organic UV filters prevent UVA and UVB rays from reaching the skin, but the similarity ends there. Organic UV filters are varying in their absorptive abilities, in that only *some* may absorb *both* UVA and UVB, while most absorb only UVB rays (Manaia *et al.*, 2013). In this way, they are reversible in their absorptive action: the same molecule may function repeatedly, as described in detail by Antoniou *et al.* (2008). Inorganic UV filters such as zinc oxide (ZnO) and titanium dioxide (TiO_2) may absorb, scatter, and/or reflect UV rays from the skin (Figure 1), so their versatile nature allows for a broader UV coverage and higher SPF labeling (Manaia *et al.*, 2013). However, ZnO provides better UVA coverage than TiO_2, and manufacturers must compromise between sunscreen transparency (pertaining to whiteness on skin) and sun protection; larger NPs better protect against UVA rays, but smaller NPs are more aesthetically pleasing with transparency (Barnard *et al*., 2016).

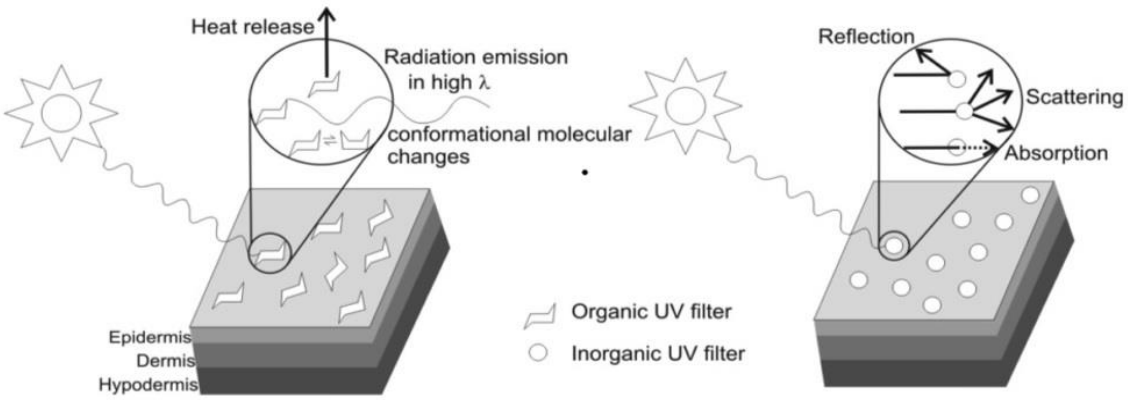


Figure 1. Action mode of organic (left) and inorganic (right) UV filters (Antoniou et al., 2008)

In recent years, TiO_2 and ZnO NPs in sun care products have received criticism for their possible adverse effects on humans and in the aquatic environment in regards to the reactive oxygen species (ROS) they produce when exposed to sunlight (*see section 3.3*) (Skocaj *et al.*, 2011; Miller *et al.*, 2012; Barnard *et al.*, 2016). Additionally, ZnO NPs are subjected to solubilization into harmful Zn^{2+} ions in seawater due to a higher pH environment (Wong *et al.*, 2010). Consequently, non-nano TiO_2 and non-nano ZnO (with nanoparticles measuring > 100 nm) are becoming increasingly popular for sunscreen

formulations produced by smaller, eco-conscious sunscreen companies (Maipas and Nicolopoulou, 2015). Interestingly, of the countries that permit the use of mineral UV filters, their "percentage limit" for the amount of a UV filter contained within a sunscreen formulation is higher compared to most chemical UV filters (20–25% or no limit for mineral UV filters versus a 10% average limit for chemical UV filters) (Table 1). However, commercial sunscreen formulas often contain a unique mixture of both physical and chemical UV filters to produce a broader spectrum of protection (Sánchez-Quiles and Tovar-Sánchez, 2015).

Table 1. Common UV filters approved in Australia (AUS), Europe (EU), Japan (JP), and United States (USA) (Osterwalder et al., 2014)

	INCI (International Nomenclature of Cosmetic ingredients)	COLIPA (Cosmetics Europe)	USAN (United States Adopted Names)	Trademark	INCI abbreviation	Form	Concentration limits in sunscreen (%)			
							AUS	EU	JP	USA
Broad-Spectrum and UVAI (340–400 nm)	Bis-ethylhexyloxyphenol methoxyphenyl triazine	S 81	Bemotrizinol	Tinosorb® S	BEMT	p	10	10	3	*
	Butyl methoxydibenzoylmethane	S 66	Avobenzone	Parsol® 1789	BMBM	p	5	5	10	3
	Diethylamino hydroxybenzoyl hexyl benzoate	S 83	–	Uvinul® A Plus	DHHB	p	10	10	10	–
	Disodium phenyl dibenzimidazole tetrasulfonate	S 80	Bisdisulizole Disodium	Neo Heliopan® AP	DPDT	p	10	10	–	–
	Drometrizole trisiloxane	S 73	Drometrizole Trisiloxane	Mexoryl® XL	DTS	p	15	15	–	–
	Menthyl anthranilate	–	Meradimate	–	MA	p	5	–	–	5
	Methylene bis–benzotriazolyl tetramethylbutylphenol	S 79	Bisoctrizole	Tinosorb® M (active)	MBBT	d	10	10	10	*
	Terephthalylidene dicamphor sulfonic acid	S 71	Ecamsule	Mexoryl® SX	TDSA	p	10	10	10	*,†
	Zinc oxide	S 76	Zinc Oxide	Z-Cote® HP1	ZnO	p, d	no limit	‡	no limit	25
UVB (290–320 nm) and UVAII (320–340 nm)	4-Methylbenzylidene camphor	S 60	Enzacamene	Eusolex® 6300	MBC	p	4	4	–	*
	Benzophenone-3	S 38	Oxybenzone	–	BP3	p	10	10	5	6
	Benzophenone-4	S 40	Sulisobenzone	Uvinul® MS40	BP4	p	10	5	10	10
	Polysilicone-15	S 74	–	Parsol® SLX	PS15	l	10	10	10	–
	Diethylhexyl butamido triazone	S 78	Iscotrizinol	Uvasorb® HEB	DBT	p	–	10	–	*
	Ethylhexyl dimethyl PABA	S 08	Padimate O	Eusolex® 6007	EHDP	l	8	8§	10	8
	Ethylhexyl methoxycinnamate	S 28	Octinoxate	Uvinul® MC 80	EHMC	l	10	10	20	7.5
	Ethylhexyl salicylate	S 13	Octisalate	Neo Heliopan® OS	EHS	l	5	5	10	5
	Ethylhexyl triazone	S 69	Octyltriazone	Uvinul® T150	EHT	p	5	5	3	*
	Homomenthyl salicylate	S 12	Homosalate	Eusolex® HMS	HMS	l	15	10	10	15
	Isoamyl p-methoxycinnamate	S 27	Amiloxate	Neo Heliopan® E1000	IMC	l	10	10	–	*
	Octocrylene	S 32	Octocrylene	Uvinul® N539 T	OCR	l	10	10	10	10
	Phenylbenzimidazole sulfonic acid	S 45	Ensulizole	Eusolex® 232	PBSA	p	4	8	3	4
	Titanium dioxide	S 75	Titanium Dioxide	Eusolex® T2000	TiO_2	p, d	25	25	no limit	25
	Tris biphenyl triazine	S 84	–	Tinosorb® A2B	TBPT	d	¶	¶	¶	¶

*Time and Extent Application (TEA), Proposed Rule on FDA approval expected not before 2014.
†Approved in certain formulations up to 3% via New Drug Application (NDA) Route.
‡Not yet approved in EU, positive opinion by Scientific Committee on Consumer Safety (SCCS).
§Not being supported in the EU and may be delisted.
¶Not yet approved in EU or anywhere else (but positive Safety Opinion on 1,3,5-Triazine, 2,4,6-tris[1,1′-biphenyl]-4-yl-, SCCS Sept/Dec. 2011).
Cosmetics Europe (formerly COLIPA): http://www.cosmeticseurope.eu/, order number shows chronology of UV filter development.
Trademarks: Tinosorb®, trademark of BASF SE, Ludwigshafen Germany; Parsol®, trademark of DSM, Kaiseraugst, Switzerland; Uvasorb®, trademark of 3V Sigma, Bergamo, Italy; Uvinul®, trademark of BASF SE, Ludwigshafen Germany; Neo Heliopan®, trademark of Symrise AG, Holzminden Germany; Mexoryl®, trademark of L'Oréal, Paris France; Z-Cote®, trademark of BASF SE, Ludwigshafen Germany; Eusolex®, trademark of Merck, Darmstadt Germany.
p, powder; l, liquid; d, dispersion.

In terms of composition, emulsifiers and emollients contribute significantly—about 30%—to sunscreen products (Osterwalder *et al.*, 2014) (Figure 2). Aside from their aesthetic purpose (consistency, durability, etc.), emollients serve to solubilize and photostabilize reactive UV filter particles (e.g., benzoate esters, octyl methoxycinnamate, avobenzone) (Osterwalder *et al.*, 2014). Organic UV filters are generally less photostable than inorganic (except for oxybenzone [Abid *et al.*, 2017]), resulting in photolysis and

harmful free-oxygen radicals that may cause allergic reactions to animals (Horio and Higuchi, 1978; Karlsson *et al*., 2009) and in some cases, carcinogenic tendencies (Gallagher *et al.*, 1984; Gasparro, 1986). Danovaro *et al.* (2008) also alludes to the potential exposure of toxic by-products from photodegraded particles

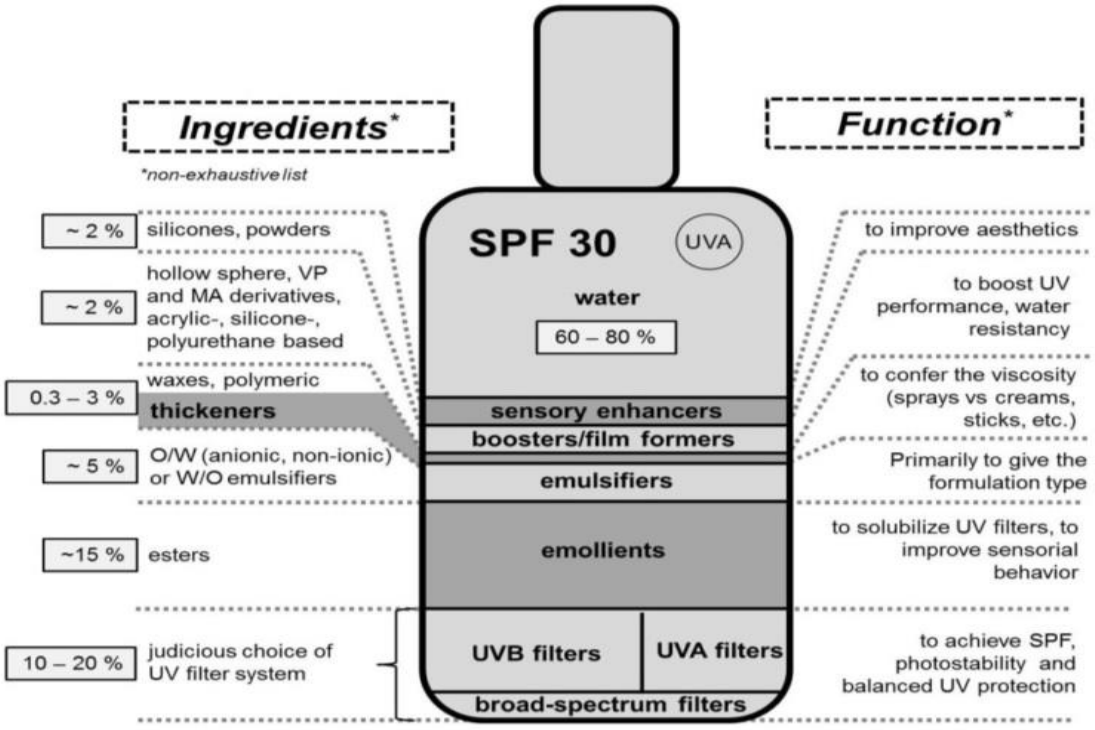


Figure 2. Composition of ingredients in an average sunscreen formula (Osterwalder et al., 2014)

in sunscreen to the marine environment, but direct studies of their toxicological effects on either humans or marine life is scarce (Nash and Tanner, 2014). Due to extremely small concentrations in seawater (pM to nM) (Sánchez-Quiles and Tovar-Sánchez, 2015), the interaction of UV filters and their by-products in aquatic ecosystems is thought to be negligible, and research is needed to clarify any effects.

1.5 Global Regulations

As with many commercial chemical products, concentration limits are often necessary to maintain low toxicity levels for consumers as well as for organisms in contaminated watersheds. Despite efforts to compromise a standard maximum of concentration percentages in sun care products, opinions on toxicity thresholds and adequate protection continue to differ both within and amongst countries (Table 1). In

Australia, UV filters are labeled as therapeutic drugs, in Canada and the United States as over-the-counter (OTC) drugs, whereas China and Europe label them as cosmetics (Osterwalder *et al.*, 2014). Not only are political views varying, but sunscreen regulation procedures occur under completely different standards across the globe.

In the United States, the integrity of sunscreen chemicals has been under the scrutinizing eye of the FDA, since new regulations passed under the Sunscreen Innovation Act (SIA) of 2014 (Printz, 2015). During this time, the US-Surgeon General called melanoma a human health crisis, yet the FDA has declined many new sun care products for the past decade (Sharfstein, 2015). Eight organic chemicals have been rejected for sunscreen use without the provision of additional data, despite Europe legalizing those same ingredients several years prior (Sharfstein, 2015). The FDA recognizes that, preceding the early 1990s, the "lack of adequate analytical methods" caused the approval of most major chemicals used in sunscreens today that would not be re-approved if analyzed by current regulatory standards (FDA, 2014). Since OTC drugs (i.e., sunscreens) in the U.S. are already categorized as "safe and effective," the regulation process to reverse the status is much slower; approval is required among several agencies in addition to economic analyses (Tucker, 2014). Minimal follow-up data for product efficacy is available for present OTC drugs, unlike prescription drugs (Tucker, 2014); once issued and approved for market, any new concerning information may take years to result in even slight rule changes (Tucker, 2014). Consequently, the FDA is extremely cautious in approving new chemical compounds.

With few toxicity studies of currently-permitted UV filters, little is known about the potential hazards of FDA-pending UV filters, and both scientists and physicians alike admit to the lack of data regarding the proposed ingredients (Printz, 2015). Cinnamates, PABAs, camphor derivatives, and phenols constitute the list of FDA-rejected UV filters; despite the lack of toxicity knowledge, exposure to these parent compounds has resulted in toxic effects in various marine studies. Specifically, one of the rejected UV filters in the United States is Ecamsule, an organic camphor derivative, patented by L'Oreal. However, it is accepted by the FDA in minute quantities (3%) from L'Oreal only (Printz, 2015). The US Public Access to Sunscreen (PASS) Coalition argues against the FDA, claiming revolutionary chemicals like Ecamsule have been commercialized in other

countries for years “without any hazardous health reports” (PASS Coalition), but Abid *et al.* (2017) demonstrated proof of Ecamsule’s instability and photodegradation similar to avobenzone (an unstable organic UV filter), while Danovaro *et al.* (2003) already demonstrated Ecamsule’s ability to increase virus production in seawater. Still, various sunscreen products do not have appropriate scientific data to prove they are completely non-hazardous to humans or the environment (Axelstad *et al*., 2013). While the currently-rejected UV filters are more photostable compared to the approved avobenzone, the majority have been deemed an “unknown” in terms of endocrine disruption or reproductive toxicity for both humans and marine life (Axelstad *et al*., 2013; Maipas and Nicolopoulou, 2015). Furthermore, the FDA states that, “sunscreens, by the very nature of their indication, define the ‘maximum use profile’” (FDA, 2014); there is no limit to the amount of sunscreen that can be used and reapplied. If apprehensions are present for human application, what could that mean for the ecosystems that become the repository for those chemicals? With the concern of toxicity for any living organism, all countries and government agencies should consider multiple vectors of chemical interactions to determine regulations (i.e., human-chemical, watershed/marine environment-chemical, and chemical-chemical interactions).

In the last decade, studies on the effects of sunscreen to the marine environment have provided enough concerning data that organizations are demanding regulation (Osterwalder *et al.*, 2014). For example, Sobek *et al.* (2013) requested that European companies put warning labels on sun care products, indicating health hazards to consumers and possible associated environmental risks to organisms in nearby coastal waters. In Europe, the Cosmetic Products Regulation (CPR) delegates marketing approval of cosmetics (including all UV filters). However, environmental risk assessments (ERAs) are not required for such products, and the EU’s regulation on classification, labelling and packaging (CLP) of substances does not include cosmetics, even though the CLP regulation’s main purpose is to “protect humans and the environment from harmful, both physical and chemical, exposures” (CLP; EC/1272/2008; Sobek *et al*., 2013). Sobek *et al.* (2013) researched all 26 currently-approved UV filters in the EU and found that 12 of them (46%) would meet the CLP classification as “hazardous to the aquatic environment” if included in the regulatory

process. But the term “hazardous” lies on a broad spectrum when discussing marine toxicology, as insufficient data and/or knowledge may often cause misinformed conclusions pertaining to differences between contaminants and pollutants.

2. Contaminants vs. Pollutants

2.1 What is the difference?

All pollutants are contaminants, but not all contaminants are pollutants. In the marine environment, a contaminant is a substance that is present in a place where it should not be, or “at concentrations above background” (Chapman, 2007), although it does not necessarily create a negative effect within its alien environment. In contrast, a pollutant is defined as a contaminant that, in addition to existing where it usually does not, produces adverse effects at a biological and even ecological scale (Chapman, 2007). Defining the difference between contaminants and pollutants is not always achievable, since current concentrations cannot be consistently and accurately measured (Stengel *et al.,* 2006); effects of the pollutant may also be too subtle to be directly measured (e.g., sub-lethal but affecting reproductive success). Primary pollutants cause negative effects on the environment they enter by their mere presence and form, whereas secondary pollutants become deleterious (albeit disputably less severe) when altered by chemical processes and other interactions (Alloway, 1997). It could be argued that nearly any substance in excess can become a pollutant, even everyday items we consume. For example, barrel loads of syrup, juice, or other foodstuffs, dumped into a body of water would surely have a negative impact on its aquatic inhabitants in high-enough concentrations (Alloway, 1997). Additionally, long-term toxicity damage to the surrounding ecosystem is not always an immediate consequence to exposure; it usually takes time to show evidence of toxicity at a larger scale (Stengel *et al.,* 2006).

2.2 Discrepancies in Science

The fine line between contaminants and pollutants is often what causes discrepancies in scientific research. Examples in literature fail to confidently distinguish either label, whether due to lack of data (Chapman *et al.,* 1996, Fent *et al.,* 2010) or dependencies on other environmental conditions that can either reduce or exacerbate the damage that might be caused by a chemical (Kusk *et al,* 2011, Miller *et al*., 2012, Yung

et al., 2015). Notably, their efforts cannot be entirely faulted; at what defining point does a contaminant become a pollutant? No scale nor chart currently exist to accurately measure marine contaminants, simply because there are too many integrated factors that affect each ecosystem and its organisms differently (Sánchez-Quiles and Tovar-Sánchez, 2015). Johnston and Roberts (2009) argue further that environmental contaminant studies are prone to overestimation if the loss of biodiversity is affected by other co-varying factors.

Presently, there is no question whether personal care products (PCPs) contaminate our oceans. In addition to toxicity exposure studies, substantial evidence of UV filter bioaccumulation within tissues of marine organisms is also available (Brausch and Rand, 2011, Bachelot *et al.,* 2012, Gago-Ferrero *et al.,* 2012, Gago-Ferrero *et al.,* 2013). UV filters have been shown to accumulate over time at levels similar to PCBs and DDT due to high environmental stability and strong lipophilicity (Brausch and Rand, 2011). However, what remains uncertain is whether current UV filter concentrations are harmful enough to marine organisms to be considered an environmental pollutant, and how the term "harmful" is considered in scientific literature. Of the few UV filter toxicity exposure studies conducted for marine organisms, some results indicate that UV filters are just "emerging contaminants of concern" but collectively fail to reach a definitive consensus due to varying external factors (Fent *et al.,* 2010) (Appendix 1).

To date, most aquatic toxicology studies are performed in laboratory settings. Controlled environments allow focus of the variables being tested, without the burden of fluctuating parameters in the natural environment interfering with results. However, this method in aquatic toxicology does not mirror the environment in which the organism resides. But conducting toxicity exposure studies on marine algae, for example, would not be efficient in the field; some organisms are too small and/or delicate to obtain accurate data without isolation. Even in laboratory settings, toxicologists may unsuccessfully define an organism's toxicity threshold (Fent *et al*., 2010). Referencing aquatic toxicology, Chapman (2007) argued that, although laboratory controls are convenient, they are "simplistic" and fail to accurately replicate and/or predict toxicity thresholds to field populations. For a better understanding of the interactions between

marine organisms and their surrounding contaminants, realistic field investigations need to be applied.

Appendix 1 compiles all marine toxicity studies to common UV filters. Their results indicate a spectrum of negative responses to UV filter exposure. The most common UV filter toxicity experiments were conducted using inorganic metal oxide nanoparticles (TiO_2 and ZnO), while marine algae were the most popular exposure subjects due to easy acquisition. From available published research, both organic and inorganic UV filters were shown to be toxic to a range of marine algae species, although their toxicity was oftentimes determined by external factors such as salinity (Aravantinou *et al.,* 2015; Yung *et al.,* 2015), light levels (Miller *et al*., 2012; Clemente *et al.,* 2014; Sánchez-Quiles and Tovar-Sánchez, 2014), and physicochemical factors like particle size and pH (Wong *et al.,* 2010; Manzo *et al.,* 2013). Other exposure subjects include corals, crustaceans, bivalves and other mollusks, annelids, echinoderms, and fishes. Seldom are studies of this nature conducted using organic UV filters, although the pilot study of this capstone project will include more of them.

Toxicological studies examine adverse chemical effects on living organisms, dose-dependent chemical relationships between organisms and their environment, and factors that influence the severity of their exposure (Díaz-Cruz and Barceló, 2015). Toxicity of UV filter exposure was determined using numerous methods, contingent on the species and UV filter being tested (Appendix 1). Observing growth rate and mortality was a common method for smaller organisms such as marine algae (Wong *et al.,* 2010; Jarvis *et al.,* 2013; Manzo *et al.,* 2013; Castro-Bugallo *et al.,* 2014) and copepods (Kusk *et al.,* 2011; Jarvis *et al.,* 2013), whereas larger organisms required more extensive assessments, such as vitellogenin analysis to assess endocrine disruption (Coronado *et al.,* 2008), gut histology to observe nanoparticle uptake (Galloway *et al.,* 2010), isotope tracing for tracking newly-accumulated UV filter particles (Buffet *et al.,* 2012), and lysosomal membrane stability to determine oxidative stress (Canesi *et al.,* 2010b; Barmo *et al.,* 2013). All studies listed were conducted either *in vivo* (using the entire animal) or *in vitro* (testing isolated cells or tissues).

3. Toxicological Effects on Marine Ecosystems

3.1 Determining Ecosystem Conditions

Before examining effects of pollution on marine ecosystems, it is important to establish a baseline condition. Defining the state of marine ecosystems in terms of "health" is neither conventional nor correct to characterize their current state. "Health" is when an organism functions optimally without evidence of abnormality or disease. To state that an ecosystem is "healthy" is merely a metaphorical comparison to organismal health (Suter, 1993). Ecosystems are not organisms and therefore do not retain the same properties or behaviors as organisms (Suter, 1993); determining the health of the organisms provides information on the condition of the ecosystem. While this metaphor is often used in applied environmental science (Suter, 1993), it should not be accepted in ecotoxicology. If health is defined by the absence of disease or abnormality, then marine ecosystems would always be "unhealthy"; latent-induced viruses and infectious bacteria continuously exist within the aquatic realm (Newman, 2009). Thus, ecotoxicology examines ecosystem conditions or indicators that may have degraded functions due to ecological instability or loss of biodiversity, but will typically represent a stable state that may or may not resemble the same stable state as before the degradation occurred (Newman, 2009). As an ecosystem changes, the organisms within it may be adversely affected by diverse biotic and abiotic pathogens, including the introduction of chemical contaminants. For example, shallow water marine ecosystems are constantly changing, at times to alternate stable states. By measuring ecosystem conditions based on ecotoxicology principles, the determination of what is detrimental to that ecosystem—in terms of causing harm to organisms that are critical to ecosystem functions—may become more apparent.

3.2 UV Filter Distribution Pathways

Marine pollution has long been recognized as a concern not only to coastal ecosystems, but amidst the pelagic and deep sea. Chemical contaminants, like many anthropogenic stressors, are not limited by physical boundaries; their potential to contaminate remote areas is a testament to UV filters' chemical resilience and ability to bioaccumulate (Díaz-Cruz and Barceló, 2015). Of the existing marine UV filter toxicity

studies, most have focused on their presence in coastal regions. However, documented cases have at least confirmed the presence of UV filters in pelagic zones of the Pacific (Goksøyr *et al.*, 2009), as well as offshore locations in the Arctic (Tsui *et al.*, 2014). No studies of the effects of UV filters or their concentrations have yet been conducted in the deep sea, although the discovery of these compounds here would not be surprising, as polychlorinated biphenyls (PCBs) are present in the deepest ocean trenches (Jamieson *et al.*, 2017). The normal concentrations of organic UV filters are measured at ng/L, while larger concentrations of μg/L are found in contaminated waters (Maipas and Nicolopoulou-Stamati, 2015). Even in these minute concentrations, sunscreen chemicals may reside within the aquatic environment for up to a century (Maipas and Nicolopoulou-Stamati, 2015), hence their bioaccumulation capability within both organisms and substrata should be determined as well as their effects.

Two pathways of chemical pollution are point and nonpoint source pollution. Point-source pollution originates from a known area and is detectable by direct measurements of the pollutants or other such evidence like mortality (Díaz-Cruz and Barceló, 2015). Examples include wastewater treatment plant (WWTP) effluents, industrial discharges, and land-based dumping of wastes, among others (Díaz-Cruz and Barceló, 2015). Nonpoint-source pollution is characterized by collective sources, such as land use and terrestrial management that negatively alter the hydrological cycles of nearby waters, producing run-off or storm-water drainage (Ritter *et al.*, 2002). Distinguishing between point- and nonpoint-source pollution is often difficult to achieve, as many contaminants received by the marine environment may already be present naturally, such as trace metals (Díaz-Cruz and Barceló, 2015).

How chemical contaminants enter the environment can be elusive; for UV filters, WWTP discharge and recreational water activities are leading pathways (Díaz-Cruz and Barceló, 2015). About 25% of sunscreen that is applied is not absorbed by the skin, and the excess is released into the surrounding water within a 20-minute period following application (Danovaro *et al.*, 2008). This contributes to the estimated 4,000–6,000 t of sunscreen potentially discharged to coastal ecosystems every year (Danovaro *et al.*, 2008), with approximately 250 t of inorganic UV filters included in that amount (Wong *et al*., 2010). The land-based removal of sunscreens through showering, laundering, or

even urinating (metabolites from kidneys contain UV filter by-products and are excreted) are sources of WWTP contamination, (Li *et al.,* 2007; Diaz-Cruz *et al.,* 2008). WWTPs are incapable of completely filtering out chemical contaminants, with a removal efficiency rate as low as 28% to 43%, according to a study in China (Li *et al*., 2007), although efficiency has improved in recent years (Margot *et al.,* 2015). Benzophenone-4 (237−1481 ng L^{-1} in Spain) (Rodil *et al*., 2008), titanium dioxide nanoparticles (<5–15 µg/L in Arizona) (Kiser *et al.,* 2009), oxybenzone (19 ng/L in New York) (Coronado *et al*., 2008), and various benzophenones and benzotriazoles (summative concentration range 104−6370 ng g^{-1} dry weight in China) (Zhang *et al.,* 2011) have all been documented from WWTP effluents, but these are examples of an exhaustive list of measurements (Ramos *et al*., 2016). Notably, these concentrations are not exclusively due to sunscreens but rather a comprehensive mixture of all PCPs and other products containing UV filters. At this source of magnitude, pinpointing which PCPs (sunscreens, soaps, etc.) are responsible for certain WWTP effluent concentrations is virtually impossible. However, seasonal spikes in UV filter concentrations from WWTPs and coastal waters have been documented, with higher concentrations usually observed throughout summer months (Plagellat *et al.,* 2006). Due to increased swimming and coastal activities during warmer seasons, one can infer the patterns are attributed to sunscreen use (Danovaro *et al.,* 2008).

3.3 Biochemical and Physicochemical Reactions of UV Filters in Seawater

PCPs can cause physicochemical and biochemical changes within marine ecosystems. Chemical contaminants are not only released into an aquatic setting that interacts with its inhabitants, but contaminants can chemically react with seawater. Two mechanisms for inorganic UV filters have gained the most attention: ROS production and dissolution of metal oxide nanoparticles (Miller *et al*., 2010) (Figure 3). Photoexcitation—electron excitation by photon (light) absorption from inorganic UV particles (TiO_2 and ZnO) under solar radiation—produces hydrogen peroxide (H_2O_2), a ROS, which has been shown to induce oxidative stress to marine phytoplankton and negatively affect their growth rate (Sánchez-Quiles, and Tovar-Sánchez, 2014). H_2O_2,

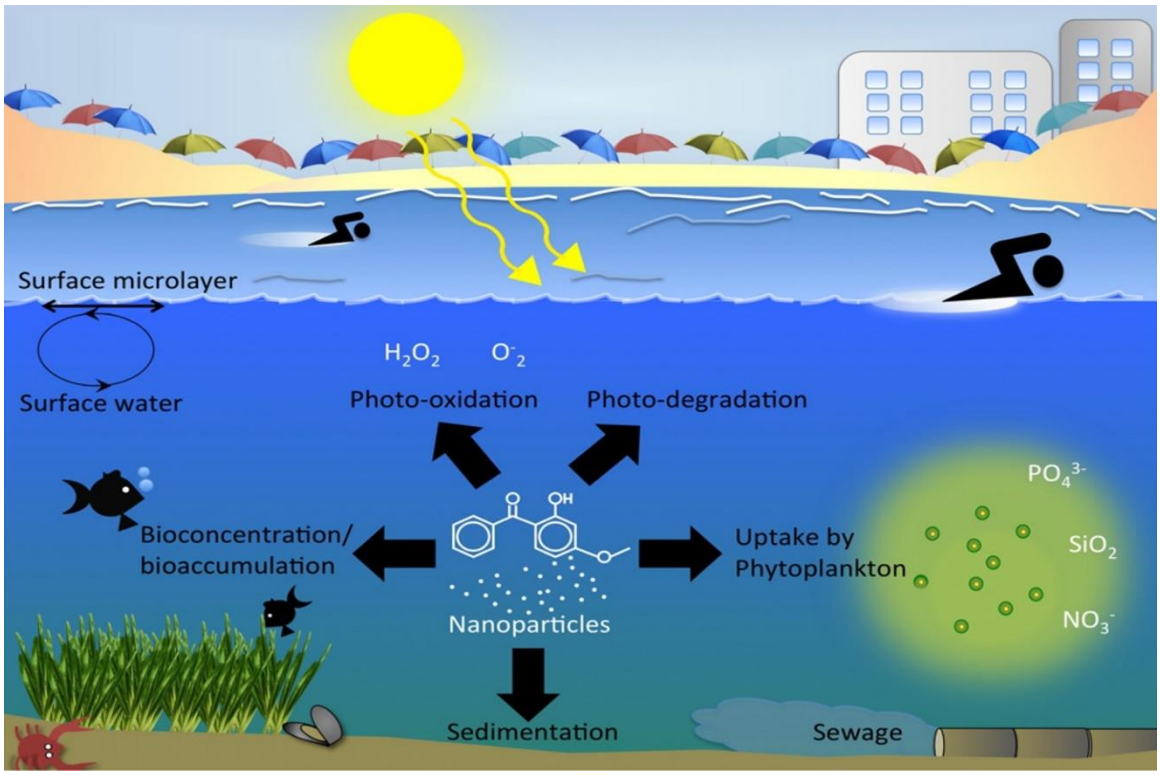


*Figure 3. Conceptual diagram transfer of sunscreen-derived products (*Sánchez-Quiles, and Tovar-Sánchez, 2015)

among all ROS produced in seawater, has the longest lifetime and highest steady-state concentrations (Lesser, 2006), so concerns of long-term effects may be justified. Nano-TiO_2 produces harmful ROS when exposed to UV radiation (Carp *et al*., 2004); their silica or alumina coating during the manufacturing process protects our skin from ROS, although ROS production is enabled once the coating dissolves in water (Lesser, 2006). ROS production does occur naturally via physicochemical processes in hydrothermal vents and biochemically via organisms' stress responses, for example, but they can damage DNA, lipids, and proteins if not removed by antioxidants (Lesser, 2006). Still, the quantity of ROS produced by engineered nanoparticles in addition to naturally-occurring ROS is of concern.

Dissolution of metal oxides in seawater introduces other issues for marine ecosystems, because ZnO and TiO_2 release Zn^{2+} and Ti^{2+} ions, respectively (Miao *et al.*, 2010). Metal oxide dissolution occurs under different physical and chemical processes in seawater than in freshwater, further complicating the ion's toxicity to and bioavailability in marine ecosystems (Baker *et al*., 2014). Metal oxide nanoparticles have a relatively

rapid dissolution rate in seawater compared to freshwater, and their solubility depends on pH and particle size (Miao *et* al., 2010; Miller *et al.*, 2010). Marine organisms are unlikely to be affected by single inorganic nanoparticles that are sized in nanometers, but it is the cumulative aggregations of Zn^{2+} and Ti^{2+} ions to larger micrometer-sized particles that may produce negative effects (Keller *et al.,* 2010). Miao *et al.* (2010) demonstrated significant growth inhibition from dissolved Zn^{2+} ions in marine phytoplankton, but not significantly from ZnO nanoparticles themselves. Miller *et al.* (2010) found that ZnO nanoparticles reduced growth rates in marine phytoplankton, although this effect was likely caused by free Zn^{2+} ions that completely inhibited uptake of manganese, a vital micronutrient for phytoplankton growth.

In addition to seawater's properties, other environmental factors can determine the fate of UV filters; sunlight photolyzes organic UV filters (see explanation on page 8), while mineral oxide nanoparticles aggregate with organic carbon found in sediments (Galloway *et al.,* 2010). Although these activities are energy-reducing by nature, it can seem misleading if the products of such reactions are not considered. For example, when the organic UV filter octyl methoxycinnamate (OMC) was degraded with both simulated and natural light, photoisomerization occurred in many products: some potentially photostable and others not photostable (MacManus-Spencer, 2011). Coupled with various chemicals in sunscreen such as emollients and emulsifiers, the instability of some UV filters makes the effect of sunscreens on marine ecosystems more elusive. Although aggregation reduces the reactivity of inorganic UV particles, it was shown to have negative effects on some marine organisms that directly interact with sediments, such as annelids (Galloway *et al.,* 2010) and bivalves (Canesi *et al*., 2010a; Libralato *et al*., 2013), but using environmentally realistic concentrations in sediments showed conflicting results (Canesi *et al*., 2010a; Buffet *et al.,* 2012). The extent of aggregation depends on various factors (size, ionic strength, pH, organic carbon content) (Dunphy *et al.,* 2006), and therefore results can vary. In summary, the biochemical and physicochemical reaction products resulting from the release of UV filters in seawater are understood, but the toxicity of their products to marine organisms requires more research on nanoparticle aggregation, dissolution, and photolysis product effects.

3.4 Cellular Reactions from Exposure to UV Filter Compounds

Current UV filter toxicity studies on marine organisms disclose that negative effects occur due to contact with these chemicals (Appendix 1), but what exactly is happening at the cellular level, and what cellular responses represent a toxic stimulus? Regardless of species and cell type, toxic compounds induce cellular stress. The type of stress experienced depends on numerous environmental factors, including species, exposure substance, temperature, pH, light, and individual fitness. Additionally, no two individuals of the same species or genotype may react identically to toxic substances at the cellular level. Some individuals will better withstand toxic exposure, and if this increases their fitness, a type of "micro-evolution" may occur resulting in organisms that are more tolerant to that substance (Medina *et al.,* 2007). Over time, these accumulated differences in sensitivity to toxic substances may then become apparent between species. Consequently, toxicity studies are difficult to conduct and to measure effects, and results will not be uniform across different phyla.

Three factors determine a chemical's toxic threshold: the chemical's structure, how much is absorbed by the organism, and the organism's ability to expel or detoxify the chemical (Understanding Toxic Substances, 1986). UV filter compounds have varying effects on cell structure and function (Appendix 1). In marine bacterioplankton, Ecamsule was found to increase virus production by inducing prophase (Danovaro *et al.,* 2003). When exposed to TiO_2 NPs, one annelid species (*Arenicola marina*) experienced DNA and cell damage, showing that TiO_2 is a genotoxicant (Galloway *et al*., 2010). Various bivalve species demonstrated signs of lysosomal oxidative stress and destabilization (Zhu *et al*., 2011; Barmo *et al.,* 2013), increased inflammatory activity (Canesi *et al.,* 2010a), and significant DNA damage in hemocytes (D'Agata *et al.,* 2014) after exposure to various forms of inorganic UV filters. Few data are available on the cytotoxicity response of crustaceans exposed to $nTiO_2$ and nZnO, but oxidative cellular stress from $nTiO_2$ was observed in brine shrimp (*Artemia salina*) under light-enhanced conditions (Clemente *et al.,* 2014), while other studies observed negative growth rates from $nTiO_2$ (Wong *et al.,* 2010; Jarvis *et al.,* 2013) and various organic UV filters (Kusk *et al.,* 2011; Paredes *et al.,* 2014). Paredes *et al*. (2014) observed growth inhibition in sea urchin larvae exposed to the chemical UV filters 2-ethyl-hexyl-4-trimethoxycinnamate

(EHMC) and 4-methylbenzylidene camphor (4-MBC), but their methodologies did not measure cellular stress responses. Marine phytoplankton, being the most well-studied specimens for UV filter toxicity, exhibited a range of cellular stress responses to both chemical and mineral UV filters, including reduced chlorophyll *a* production and fluorescence (Miao *et al.,* 2010; Castro-Bugallo *et al.,* 2014; Sánchez-Quiles and Tovar-Sánchez, 2014; Hazeem *et al.,* 2016; McCoshum *et al.,* 2016), oxidative stress (Wong *et al.,* 2010; Miller *et al.,* 2012; Castro-Bugallo *et al.,* 2014; Sánchez-Quiles and Tovar-Sánchez, 2014; Suman *et al.,* 2015; Xia *et al.,* 2015), reduced cellular division rates (Peng *et al.,* 2011), decreased cellular integrity (Miller *et al.,* 2012; Wang *et al*., 2016), decreased cellular concentration-response functions (Manzo *et al.,* 2013), decreased cellular viability (Suman *et al.,* 2015), and reduced enzymatic activity and lipid peroxidation (Xia *et al.,* 2015). Some marine phytoplankton are more negatively charged, allegedly attracting more free metal ions and potentially causing greater toxicity (Wong *et al.,* 2010).

Physiological and molecular stress responses in corals can be demonstrated through various mechanisms, depending on life stage and species (Morgan *et al*., 2001). Coral colonies are particularly sensitive to chemical contaminants due to their thin (about 100 µm), outer, lipid-dense tissue covering the skeleton that may facilitate uptake of certain lipophilic UV filters (Peters, 1997). When conducting individual coral toxicity assays on the effects of UV filters, characteristics of stress may include: expulsion of symbiotic zooxanthellae and mucous production (Danovaro *et al*., 2008), endocrine disruption (Downs *et al.,* 2016), functional and structural cell failure and necrosis (Downs *et al.,* 2014), and larval settlement inhibition (Downs *et al.,* 2014; Sharp *et al.,* 2015; Downs *et al.,* 2016). More specifically, scleractinian coral toxicity studies have shown evidence of significant coral bleaching (Danovaro *et al*., 2008) and zooxanthellae expulsion in mature fragments (Jovanović and Guzmán, 2014) as well as coral planulae (Downs *et al*., 2014; Downs *et al*., 2016) when exposed to varying concentrations and types of UV filters. In planulae studies, larval settlement was inhibited with increased amounts of benzophenone-2 and 3 (BP-2 and 3) (Downs *et al*., 2014; Sharp *et al*., 2015; Downs *et al*., 2016), while multiple sunscreen formulas induced viral lytic cycles in coral nubbins' zooxanthellae (Danovaro *et al.,* 2008). With only a handful of coral studies to

reference, it is imperative that more research is conducted to understand the effects of chemical pollution.

4. Challenges of Marine Toxicology

4.1 Difficulties in Measuring Marine Pollutants

Marine toxicology is challenging due to the need for substantial funding and availability of sensitive equipment, unavoidable variability in samples and exposure subjects, sub-par methodologies, and lack of research and data. Even with reliable quantitative data, it is still unknown where some pollutants originate and how (or if) negative effects on marine ecosystems from these pollutants may be reversed. Additionally, new toxic substances are continuing to be discovered that were either previously unknown or never recognized as a pollutant until now. Regardless, new sources of contamination in marine ecosystems should be researched, especially when the source is one of the fastest-growing markets in the world: sunscreen.

Detection of organic UV filters in the marine environment is an extensive process. Due to their extremely low concentrations in seawater (pM–nM), a pre-concentration/extraction step is required before the final analysis of trace-level organic compounds (Ferrera *et al.,* 2004); this requires using sensitive methods such as analyte isolation and enrichment that can be applicable to soil, sediment, and seawater. (Ferrera *et al.,* 2004). Inorganic nanoparticle analysis uses various techniques that provide useful information about their properties, such as separation methods (size distribution), electron microscopy (morphology), scattering (concentration), and spectroscopy (crystallographic structure) (Sánchez-Quiles and Tovar-Sánchez, 2015). Despite descriptive analyses, the quantification of UV filters in the marine environment is still limited due to the inadequacies of current methods and changes in contaminant concentrations depending on location and coastal currents.

Methodologies in UV filter toxicity, especially inorganic NPs, are inconsistent due to varying experimental designs from trial to trial, making it difficult to compare results (Schrurs and Lison, 2012; Juganson *et al.,* 2015). Knowledge of how TiO_2 and ZnO NPs can negatively affect the marine environment is therefore lagging behind recent advancements in nanotechnology (Juganson *et al.,* 2015). New techniques have recently

been developed for analysis of inorganic UV filter NPs, although results of these analyses in seawater are scarce (Sánchez-Quiles and Tovar-Sánchez, 2015).

4.2 Suggested Technological/Scientific Advancements and Studies

While measuring chemical contaminants in minute concentrations has been a recent technological advancement, there is still much to be improved. As stated, toxicology research is costly, and decisions in experiments of this nature must be made carefully. For example, the number and types of chemical analyses that can be made, which organisms to study and endpoints to be measured, tradeoffs to be made in concentrations, and periods of exposure to be tested are all necessary to consider with limitations in funding. Nevertheless, there are still untapped outlets of toxicology research and environmental legislation that could be investigated without extensive funding, such as creating toxicity models, conducting toxicity assays of untested chemicals, establishing uniform methodologies, enacting stricter legislation, and increasing WWTP removal efficiency, to name a few.

Nanotechnology is a rapidly-advancing sector of the biotech world. To avoid unnecessary costs, perhaps developing a model based on available toxicity data is a beginning approach. For engineered nanomaterials (ENMs) such as TiO_2 and ZnO used in inorganic sunscreens, Juganson *et al.* (2015) recently created a database, NanoE-Tox, with existing nano-ecotoxicological information that could be useful in toxicity models (i.e., quantitative [nano]structure-activity relationships, or QSARs/QNARs). If developed, these models could illustrate and "predict toxicity mechanisms of ENMs based on their physio-chemical properties" (Juganson *et al.*, 2015). Although this database provides data for ENMs and would therefore only offer insight on mineral-based sunscreen toxicity, models and databases for organic chemicals could conceivably follow in the future.

The lack of UV filter toxicity research, especially for chemical UV filters, is still a problem. As of 2008, there were 50 organic and inorganic compounds permitted internationally (by different legislations) to use as UV filters in commercial sunscreens, yet only 16 have been analyzed in marine toxicity assays (Sánchez-Quiles and Tovar-Sánchez, 2015). Fortunately, there is growing interest in sunscreens' effects on marine

ecosystems, which is stimulating research within both aquatic and terrestrial sectors (Aravantinou *et al.*, 2015; Chen *et al.*, 2017; Abid *et al.*, 2017). For example, terrestrial plants, like scleractinian corals, have also been shown to activate defense systems in response to oxybenzone exposure (Chen *et al*., 2017). Additionally, high concentrations of ZnO and TiO_2 NPs were shown to reduce soil bacteria community diversity (Ge *et al.,* 2011). With the continuation of more research in a novel field, patterns in data may become more apparent that were previously overlooked.

Since 2015, legislation has been proposed in areas such as Hawaii and Europe to ban one of the most common UV filters, oxybenzone. While oxybenzone is only one type of chemical UV filter, it is also used in many other PCPs and plastics, and it has therefore received substantial media attention (Downs *et al.,* 2016). Due to Downs *et al.*'s (2016) research, Hawaiian legislators approved a ban on oxybenzone in April 2018, but it could be stalled by sunscreen manufacturing companies that are demanding more research. In such cases of a rapidly-growing product market, consumers will ultimately buy what is cheapest and most effective in the short-term. If consumer awareness cannot successfully compete against market prices of cheaper (yet more harmful) sunscreens, then perhaps a direct approach to ban certain substances is a more realistic solution.

The release of chemicals through point-source pollution such as WWTP effluents contributes significantly to chemical pollution. The removal efficiency of unwanted chemical contaminants from WWTP facilities is surprisingly high, (generally >70%), with some UV filters being removed by over 90% (Margot *et al.,* 2015). However, marine life may still be affected by UV filter discharge despite efficient removal techniques (Margot *et al.,* 2015); marine organisms have shown biological stress with UV filter concentrations as low as 10 µL (Danovaro *et al.,* 2008). Optimizing conventional treatments and creating more advanced treatments in WWTPs for lipophilic UV filters that are difficult to remove (e.g., octocrylene) may help to further increase removal efficiency (Margot *et al.,* 2015).

5. Pilot Study by Coral Restoration Foundation

5.1 Study Interest and Background

The Coral Restoration Foundation (CRF) based in Key Largo, Florida, is only one of multiple organizations dedicated to restoring the abundance and protecting the resiliency of tropical coral reefs. Using propagation techniques, their corals are grown in offshore nurseries until they are mature enough to be transplanted onto reefs, initiating a human-mediated recovery process (Coral Restoration Foundation, 2017). CRF's target coral species is the staghorn coral (*Acropora cervicornis*), since it has severely declined during the past 30 years (by 80%), earning a "threatened" status on the United States Endangered Species List in 2006 (FWS, 2006) and a "critically endangered" assessment in 2008 (IUCN, 2017). This practice of active restoration has gained popularity in the last 20 years, due to anthropogenic activity inhibiting natural coral recovery rates (Rinkevich, 2005). Active coral restoration has proven to be effective as technology and methodologies improve (Boch and Morse, 2012; Young *et al.,* 2012; Xin *et al.,* 2016), but there are many unknown implications to coral restoration success (Ware, 2015). Chemical contaminants such as sunscreens may directly and/or indirectly interfere with the coral restoration process.

In 2014, CRF noticed a group of *A. cervicornis* fragments dying in their Tavernier Nursery after being handled by an individual diver. They suspected this volunteer had sunscreen on his/her hands prior to entering the water. Consequently, CRF decided to initiate a pilot study testing sunscreen exposure to their own *A. cervicornis*. When divers are working in the nurseries, corals are handled most often with bare hands that may or may not have been exposed to sunscreen formulas. It is important to note that coral fragments do not usually die after handling alone, since CRF staff have used these handling procedures for many years with success. Therefore, it was hypothesized that corals in the CRF nursery are more susceptible to dying after being handled by divers using certain types of sunscreen.

5.2 Methods

The study was conducted on July 23, 2015, at the CRF's Tavernier Nursery located at 24◦58' 55.60" N, 80◦26' 12.11" W. Before the experiment, each handler

liberally applied one of the ten respective sunscreen formulas on both the front and back of their hands (Table 2). After allowing the sunscreens to dry for at least five minutes, the handlers dove down to the CRF Tavernier Nursery using open circuit SCUBA. At the bottom, handlers knelt in the sand in a semicircle behind their set of fragments, which had been cut by Ken Nedimyer from the same genotype (K2). The divers gently picked up and loosely held one coral fragment in each hand for one minute to ensure ample time for the coral fragments to be exposed to the sunscreen. After this period, the handlers manipulated the coral fragments as is normally done, inserting a loop of monofilament line around one end and tightening it to securely hold the fragment and using pliers to clamp down on a lead crimp so it would not slip out of the monofilament loop. One by one in order, each handler carried their prepared fragments to a new nursery tree that had been made from polyvinyl chloride (PVC) pipe. Fragments were hung on the tethered PVC tree "branches" by inserting the free end of each fragment's monofilament line through a bored hole on the branch and clamping down on a lead crimp to keep it attached. The PVC tree floated upright so that the fragments were located approximately 20 feet below the surface but above the sand bottom at about 30 feet. Control fragments were handled by Ken Nedimyer with the same methodologies, however without sunscreen-laden hand exposure. Each branch contained both treatment and control fragments; treatment corals were hung on one side of the PVC tree "trunk", and control fragments on the other side, with a total of 10 branches (5 treatment and 5 controls on each branch for each sunscreen brand) (Figure 3). An equal number of treated and control coral fragments were hung on each branch (n = 5).

Table 2. Sunscreen formulas used in case study

Mineral (inorganic) Sunscreens					
Treatment Number	**Brand Name**	**SPF**	**UV Filters**	**Water Resistance**	**Claims**
1	Stream2Sea	20	Titanium Dioxide 6.6% (Non-nano)	80 min.	Biodegradable, Eco/Reef Safe
2	3rd Rock Sunblock	30+	Zinc Oxide 23.5% (Non-nano)	N/A	Eco/Reef Safe
3	Raw Elements	30	Zinc Oxide 23% (Non-nano)	80 min.	Eco/Reef Safe
4	Artistry	50+	Zinc Oxide 12.66% Oxtinoxate 6.8% Octisalate 4.5% Titanium Dioxide 2.49%	N/A	N/A
5	Neutrogena (Skin Sensitive)	60+	Titanium Dioxide 4.9% Zinc Oxide 4.7%	80 min.	N/A

Chemical (organic) Sunscreens					
Treatment Number	**Brand Name**	**SPF**	**UV Filters**	**Water Resistance**	**Claims**
6	Reef Safe	45+	Octocrylene 8.0% Octinoxate 7.5% Oxybenzone 6.0% Octisalate 5.0% Homosalate 5.0%	80 min.	Non-Toxic to Sea Life
7	Equate Sport	50	Homosalate 13% Oxybenzone 6.0% Octisalate 5.0% Octocrylene 5.0% Avobenzone 3.0%	80 min.	N/A
8	Sun Bum	50	Homosalate 10% Oxybenzone 6.0% Octisalate 5.0% Avobenzone 3.0% Octocrylene 2.75%	80 min.	N/A
9	Coppertone Water Babies	70+	Homosalate 15% Octocrylene 10% Oxybenzone 6.0% Octisalate 5.0% Avobenzone 3.0%	80 min.	N/A
10	Coppertone Ultra Guard	70+	Homosalate 15% Octocrylene 10% Oxybenzone 6.0% Octisalate 5.0% Avobenzone 3.0%	80 min.	N/A

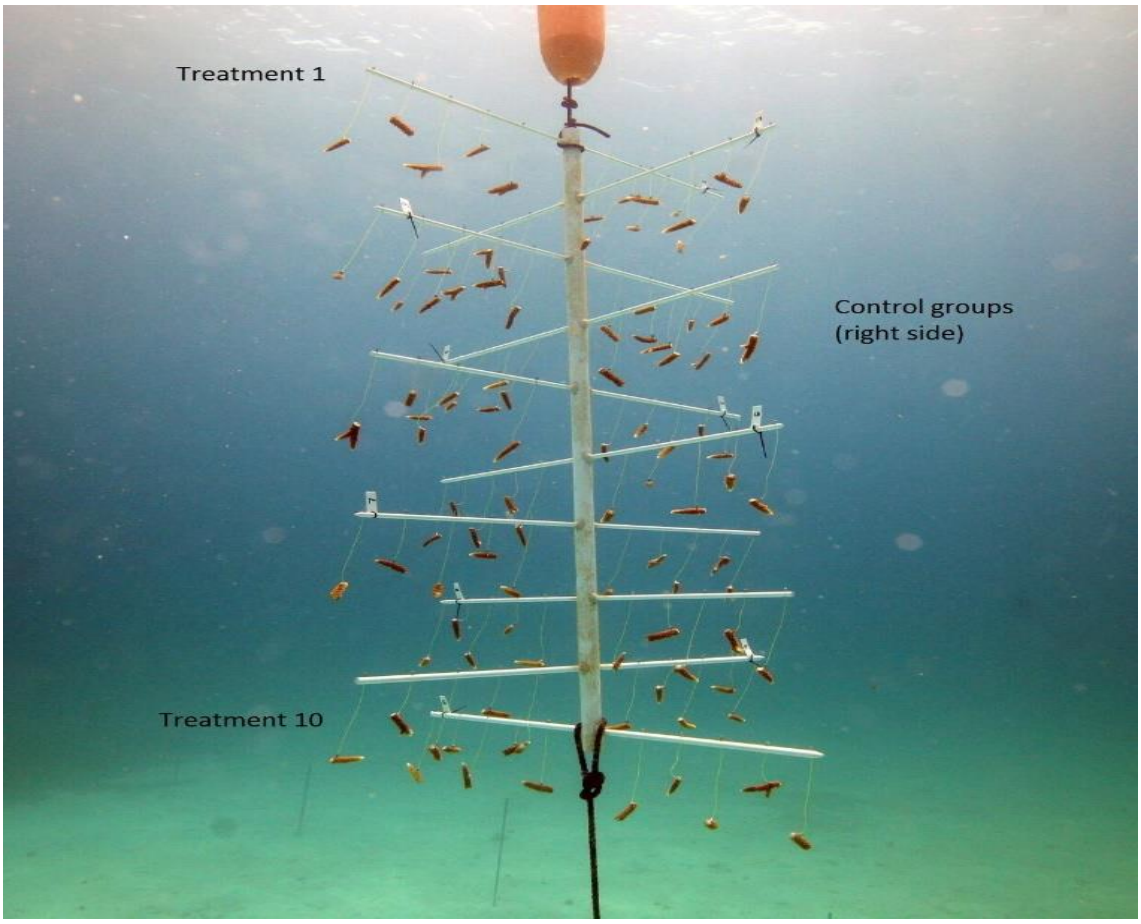


Figure 4. CRF coral nursery "tree." Control fragments were hung on one side of the trunk, whereas fragments treated with sunscreen were hung on the other side.

Coral fragments were collected by divers 10 days after the exposure to various sunscreen formulas in the CRF Tavernier nursery. All fragments appeared to have 0% visual tissue loss at the time of collection. During collection, two treated coral fragments and two control fragments from each treatment were clipped from their monofilament lines at 5-cm lengths using diagonal cutters, giving a final sample size of n = 2 instead of the original n = 5. Fragments were placed in plastic centrifuge tubes with ambient seawater that were labeled with the corresponding treatment number (1–10). Samples were then brought up to the boat, where they were immediately fixed using a formaldehyde-based solution of Z-Fix Concentrate (1 part, from Anatech, Ltd.), diluted with ambient seawater (4 parts) for preservation in plastic centrifuge tubes labeled with the corresponding treatment number, capped tightly, and sealed with Parafilm for

transport. The fixed samples were taken to George Mason University's Histology Laboratory for histoslide preparation.

Each sample was photographed and the photographs were compiled in a Word document to form trim sheets (Appendix 2). Samples were trimmed into approximately 2-cm long fragments using a Dremel tool and a diamond-coated tile-cutting blade. On the image of each sample, the location of every cut was marked to denote subsamples. Each sample was cut into 3–4 subsamples, depending on size, and the corresponding numbers of the subsamples were marked on the trim sheets.

Fixed coral fragments were processed into histoslides using the procedures described in Miller *et al.* (2014). Subsamples were decalcified using 10% disodium ethylenediaminetetraacetic acid (EDTA) at pH 7, changing the solution every 24–28 h. Following decalcification, subsamples were rinsed in tap water for approximately 30 minutes, trimmed into 2–3 mm slices, then placed in tissue cassettes and stored in 70% ethanol. Cassettes were then processed through a graded series of ethanols (70%, 80%, 95%, 100%), cleared and infiltrated with molten Paraplast Plus®, then embedded in Paraplast Xtra® (Peters *et al.,* 2005). Sections were then mounted on microscope slides, stained (with Harris's hematoxylin and eosin, and Giemsa for Gram-negative microorganisms), and coverslipped with Permount™ mounting medium (Miller *et al.,* 2014).

Histoslides were examined without knowing the treatment condition (i.e., blind) using light microscopy in the Halmos College of Natural Sciences and Oceanography's Histology Laboratory and their condition evaluated according to criteria developed by Dr. Peters (Appendix 3) and modified in consultation with her during the summer of 2017. Photomicrographs of histoslides were taken using an Olympus BX43 microscope with attached DP-2 camera. Relative condition parameters (e.g., tissue architecture, cellular integrity, zooxanthellae abundance, pathological changes) received a semi-quantitative score based on severity of tissue changes ranging from 0 –5 (0 = Change Not Present, 1 = Minimal Change, 2 = Mild Change, 3 = Moderate Change, 4 = Marked, 5 = Severe Change) (Miller *et al.,* 2014).

Condition parameter scores for apparently healthy and sunscreen-exposed coral samples from each of the 10 treatments were compared using standard statistics and two-

tailed t-tests with unequal variance. Tissue degradation scores for all the subsamples of a fragment were averaged together first to calculate mean scores for each respective sample's parameters. Sunscreen brands were grouped by UV filter type (chemical or mineral) as opposed to individual sunscreen brands due to the small sample size (n = 2) to compare with other studies.

5.3 Results

No significant differences were found between control and treatment mean scores for any of the examined parameters (Figures 5–8). However, chemical treatments had significantly higher scoring for mesenterial filament RLOs (rickettsia-like organisms that are obligate intracellular parasites) (T-test, $p = 0.015$) and costal tissue loss (T-test, $p = 0.039$) for $p < 0.05$, indicating that mesenterial filament RLOs were more numerous and costal tissue loss was more severe in chemical treatments versus mineral treatments (Figure 7). However, these significant p-values did not affect the overall significance of chemical versus mineral treatments. No other mean condition parameter scores produced significant p-values in any comparison. When comparing all treatments against controls, the averages were generally equal (Figure 8).

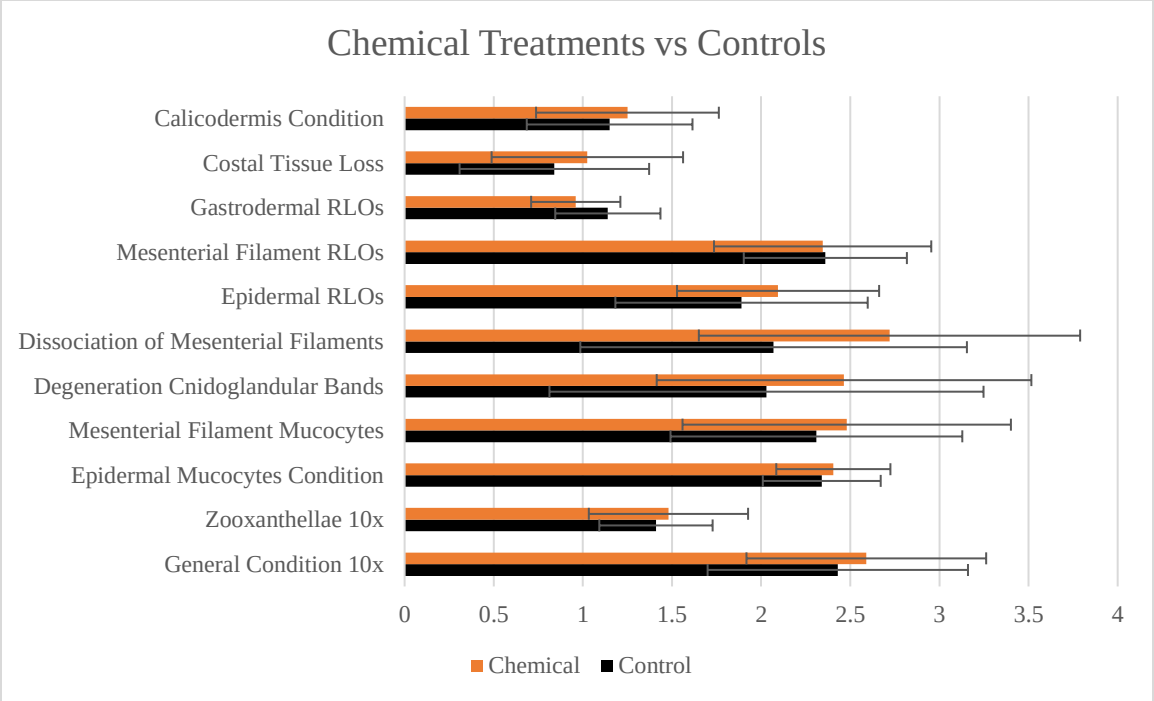


Figure 5. Average scoring of chemical sunscreen formula treatment and control treatment condition parameters

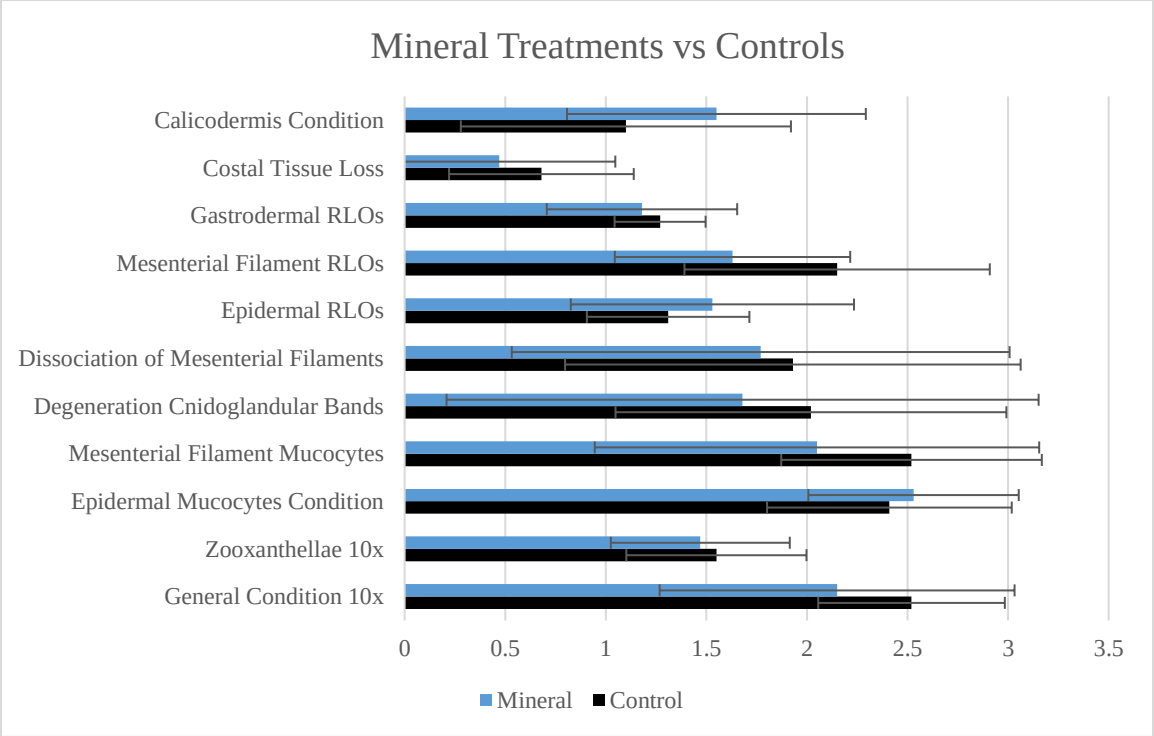


Figure 6. Average scoring of mineral sunscreen formula treatment and control treatment condition parameters

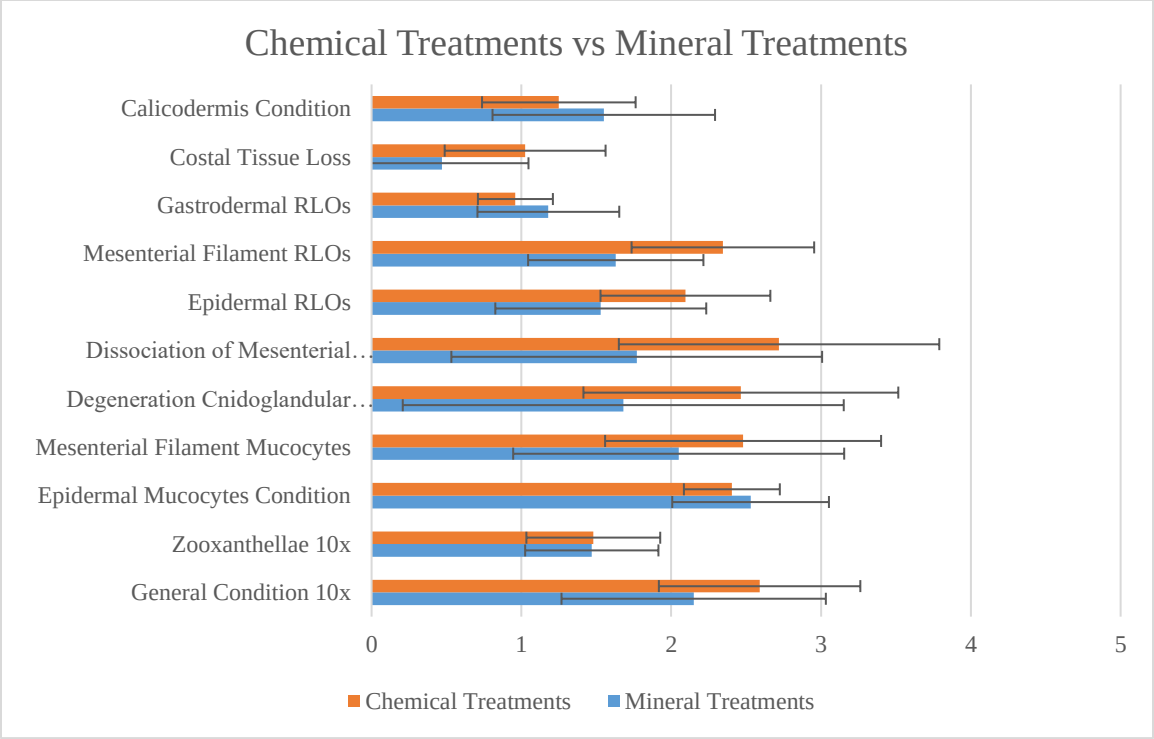


Figure 7. Average scoring of chemical sunscreen formula treatment and mineral sunscreen formula treatment condition parameters

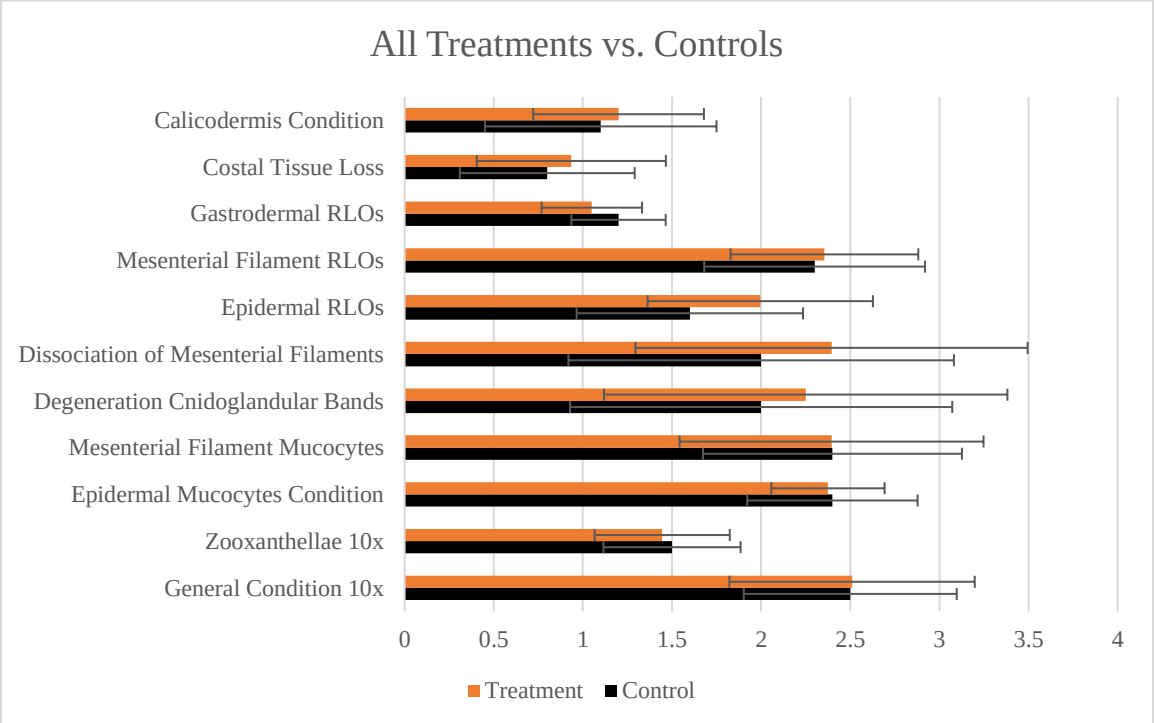


Figure 8. Average scoring of all sunscreen formula treatments and all control treatment condition parameters

Both treatment and control samples demonstrated hypertrophied epidermal mucocytes (i.e., mucus production and release) in some foci (Figures 9a–c). Ingested planktonic remnants were observed around tentacles and cnidoglandular bands in the gastrovascular cavities of treated (9 total) and untreated (10 total) samples, showing that food intake persisted despite sunscreen exposure (Figures 10a–c). Actinopharynx structure for both chemical and mineral treatments retained general integrity with flagellated supporting cells visible along the body wall (Figures 11a–b). Condition of cnidoglandular bands and mesenterial filaments within the gastrovascular cavity were not significantly different between chemical and mineral samples (Figures 12a–b). Additionally, nearly all samples showed division of zooxanthellae in the surface body wall gastrodermis (Figures 13a–c), demonstrating cell growth with no visual signs of tissue loss or zooxanthellae expulsion ("bleaching") at the time of collection (Appendix 2).

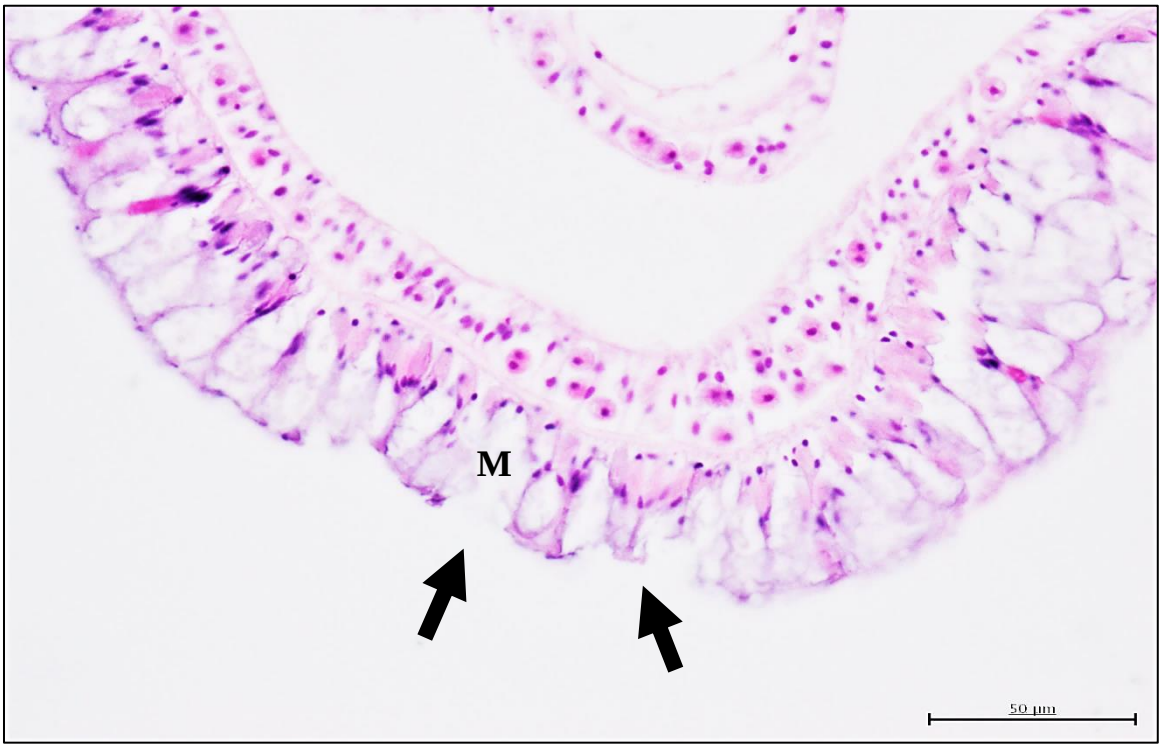


*Figure 9a. Hypertrophied mucocytes (***M***) in surface body wall of control sample with mucus release*

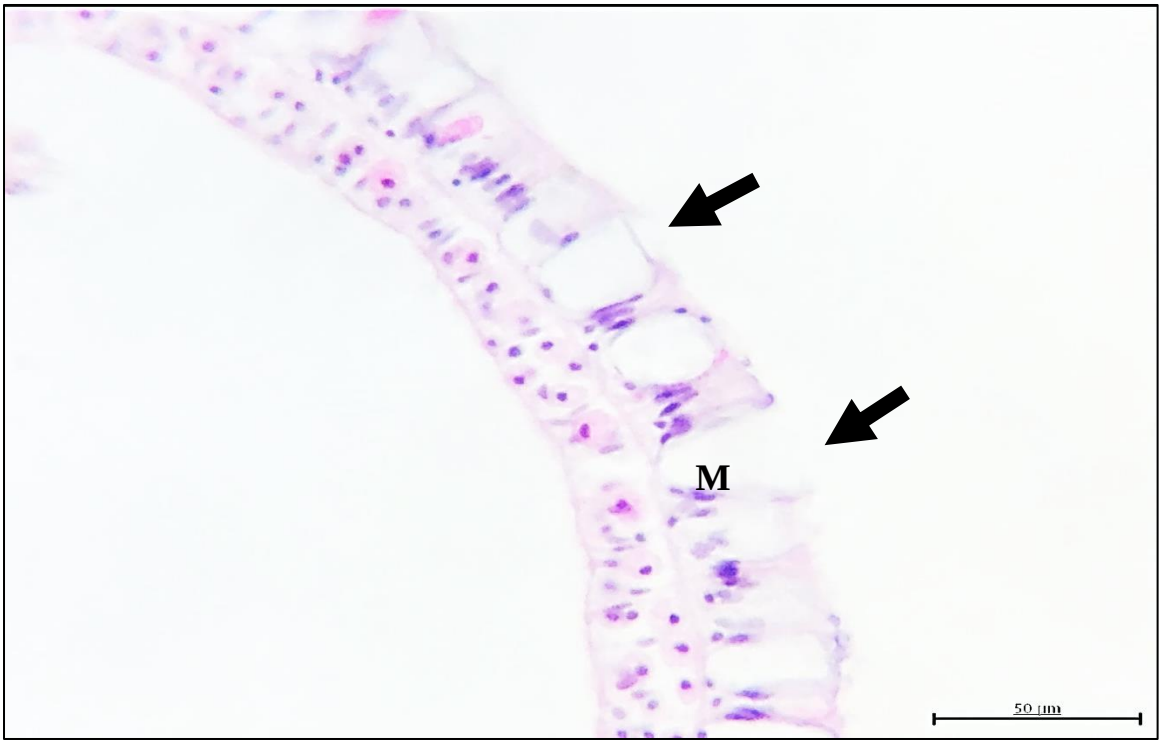


*Figure 9b. Hypertrophied mucocytes (**M**) in surface body wall of mineral sample with mucus release*

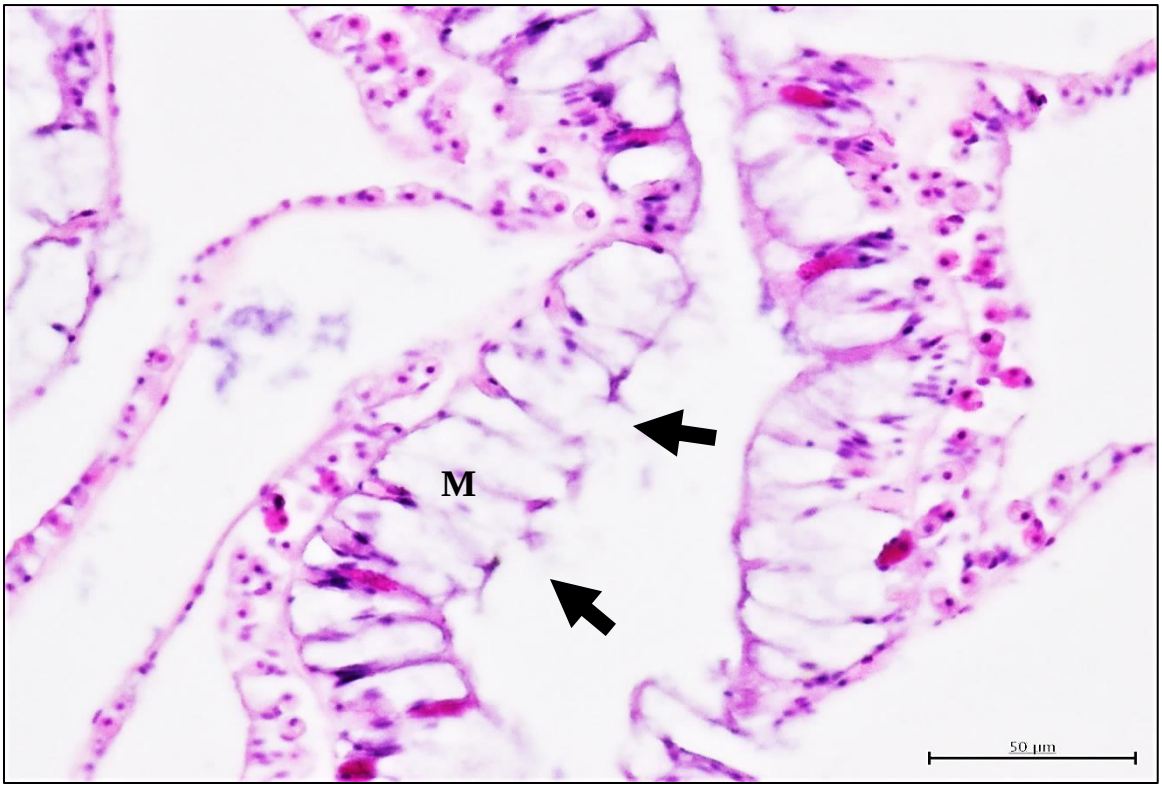


*Figure 9c. Hypertrophied mucocytes (**M**) in surface body wall of chemical sample with mucus release*

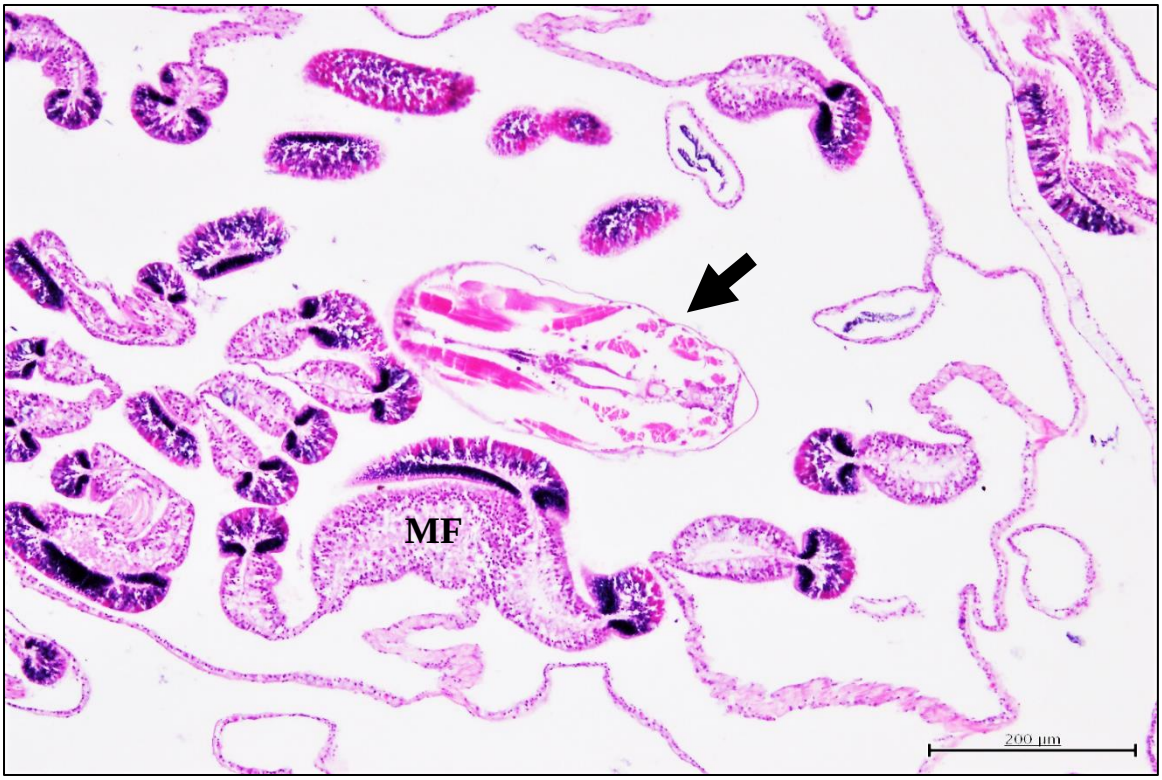


*Figure 10a. Ingested plankton surrounded by mesenterial filaments (**MF**) in control sample*

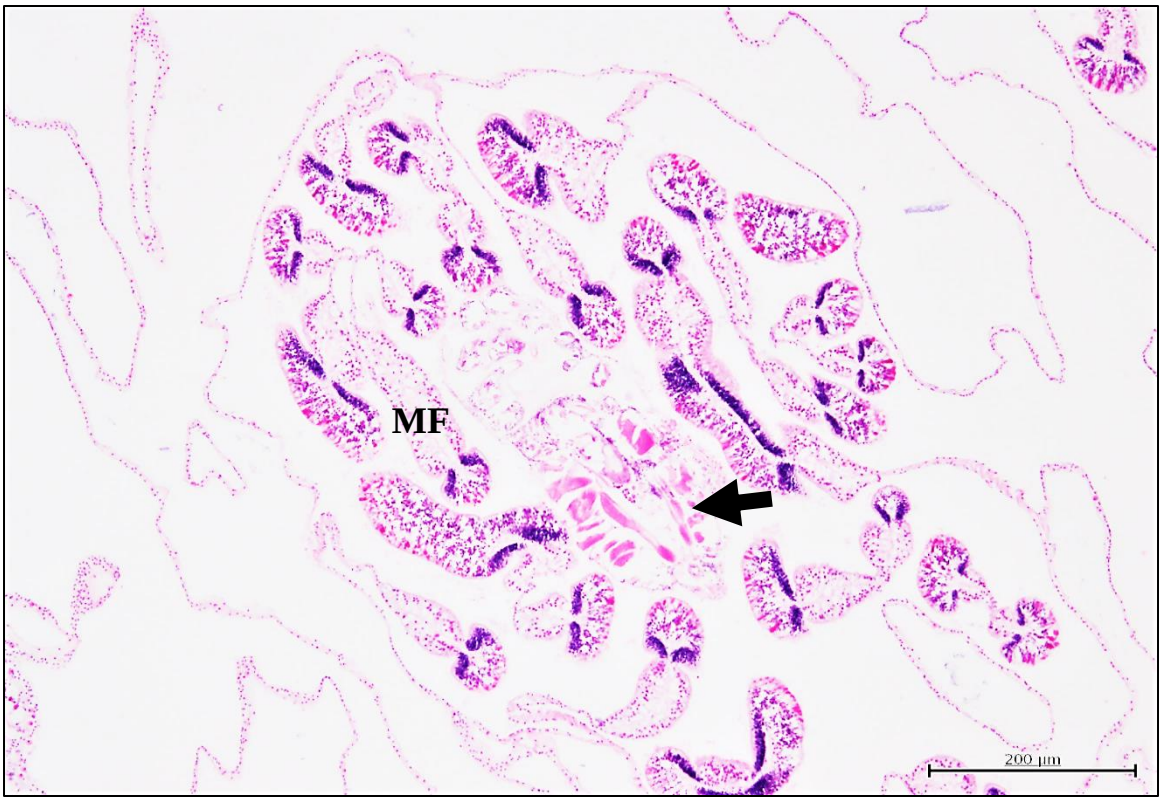


*Figure 10b. Ingested plankton surrounded by mesenterial filaments (**MF**) in mineral sample*

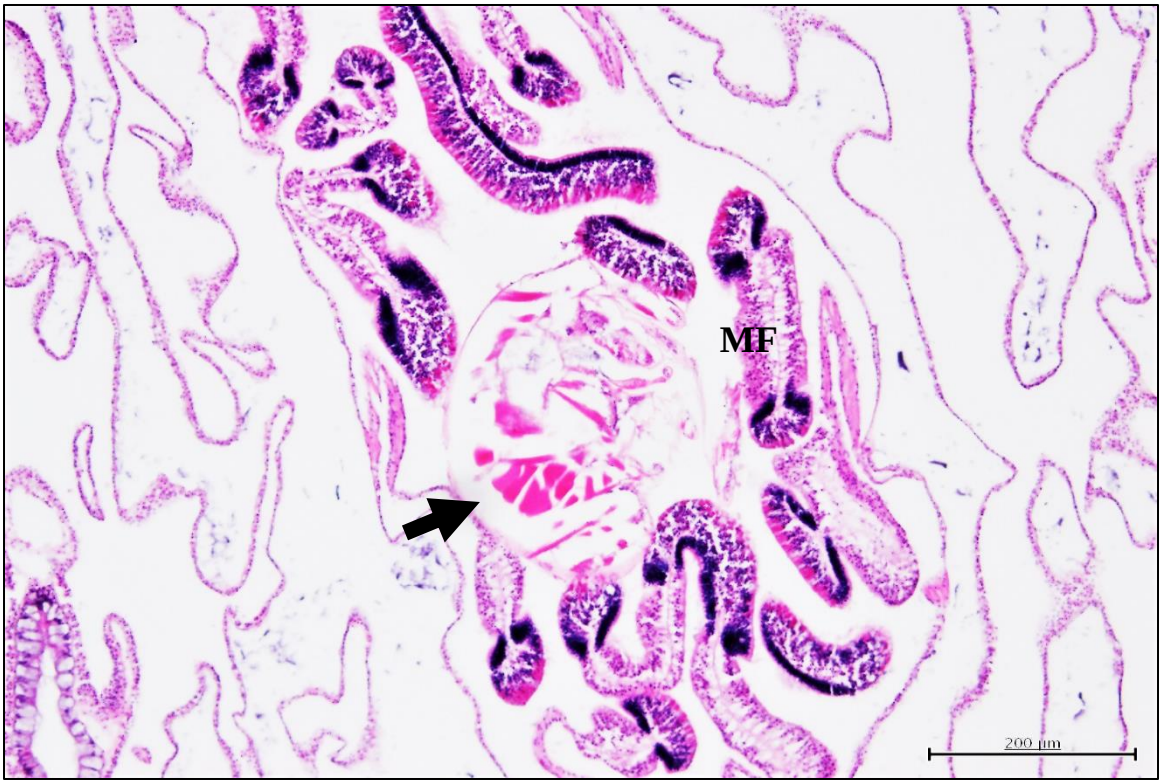


*Figure 10c. Ingested plankton surrounded by mesenterial filaments (**MF**) in chemical sample*

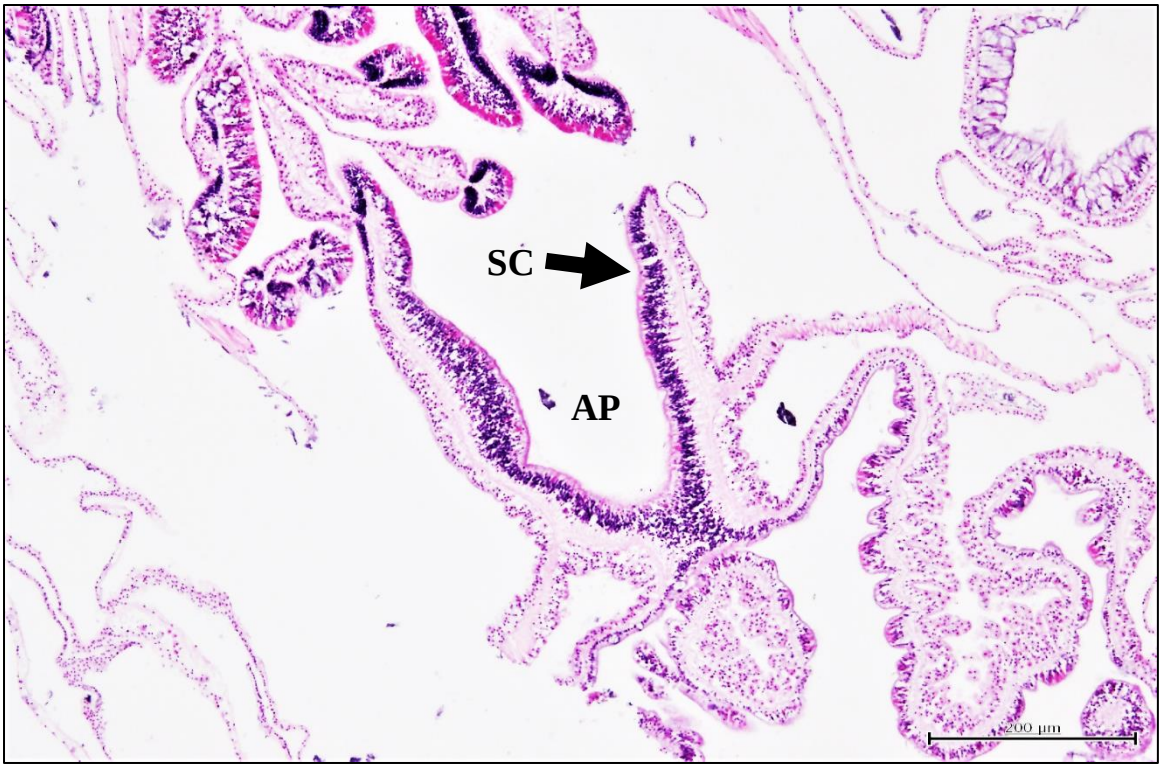


*Figure 11a. No tissue anomalies in actinopharynx (**AP**) with healthy, flagellated supporting cells (**SC**) along the body wall in mineral treatment sample*

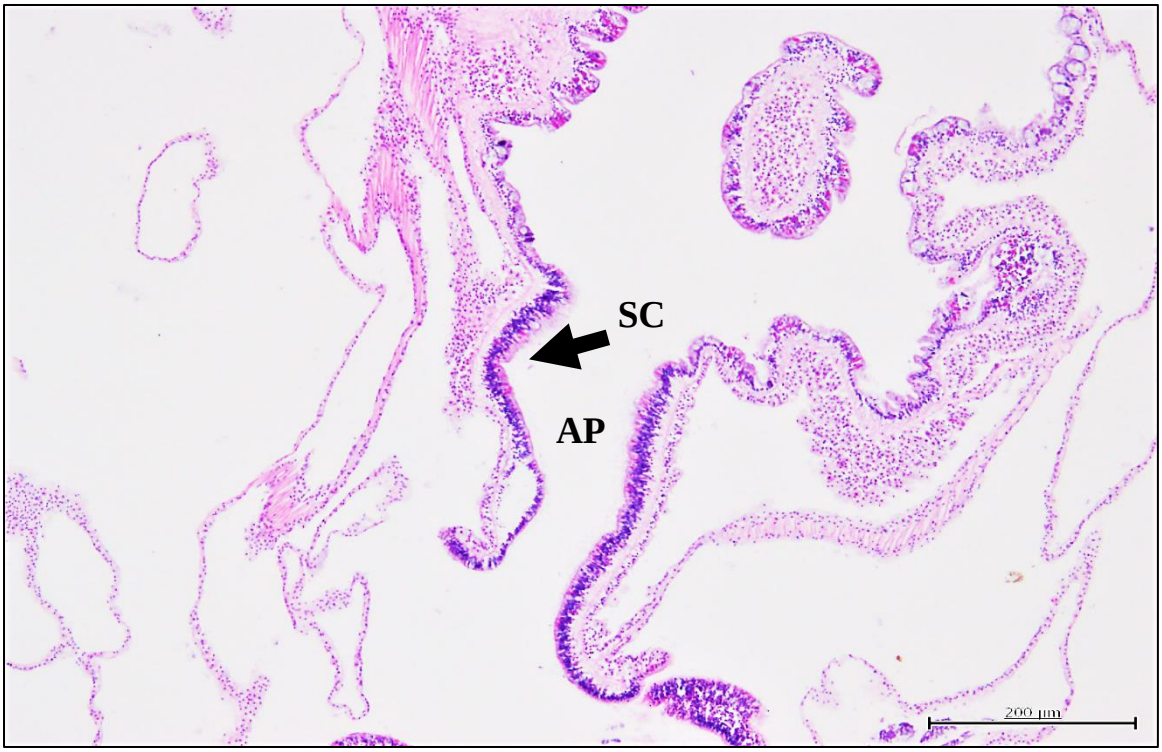


*Figure 11b. No tissue anomalies in actinopharynx (**AP**) with healthy, flagellated supporting cells (**SC**) along the body wall in chemical treatment sample*

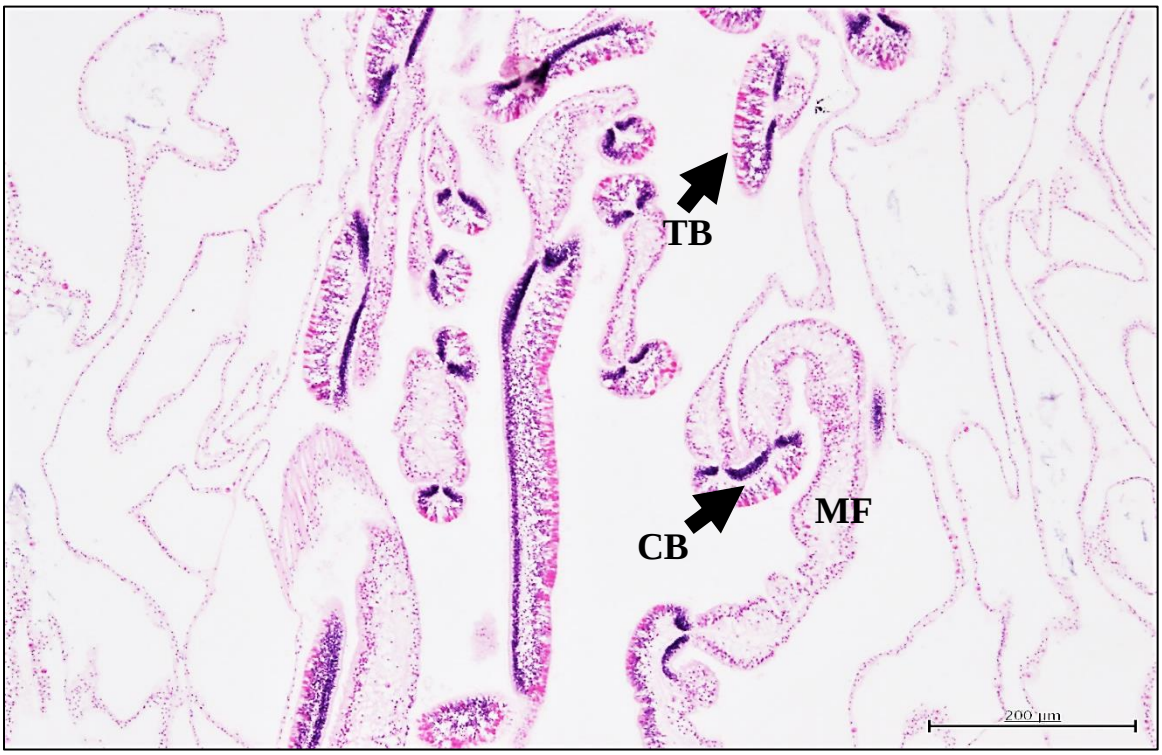


*Figure 12a. Apparently-healthy mesenterial filaments (**MF**) with cnidoglandular bands (**CB**) on the free edge with terminal bars (**TB**) well-formed in mineral treatment sample*

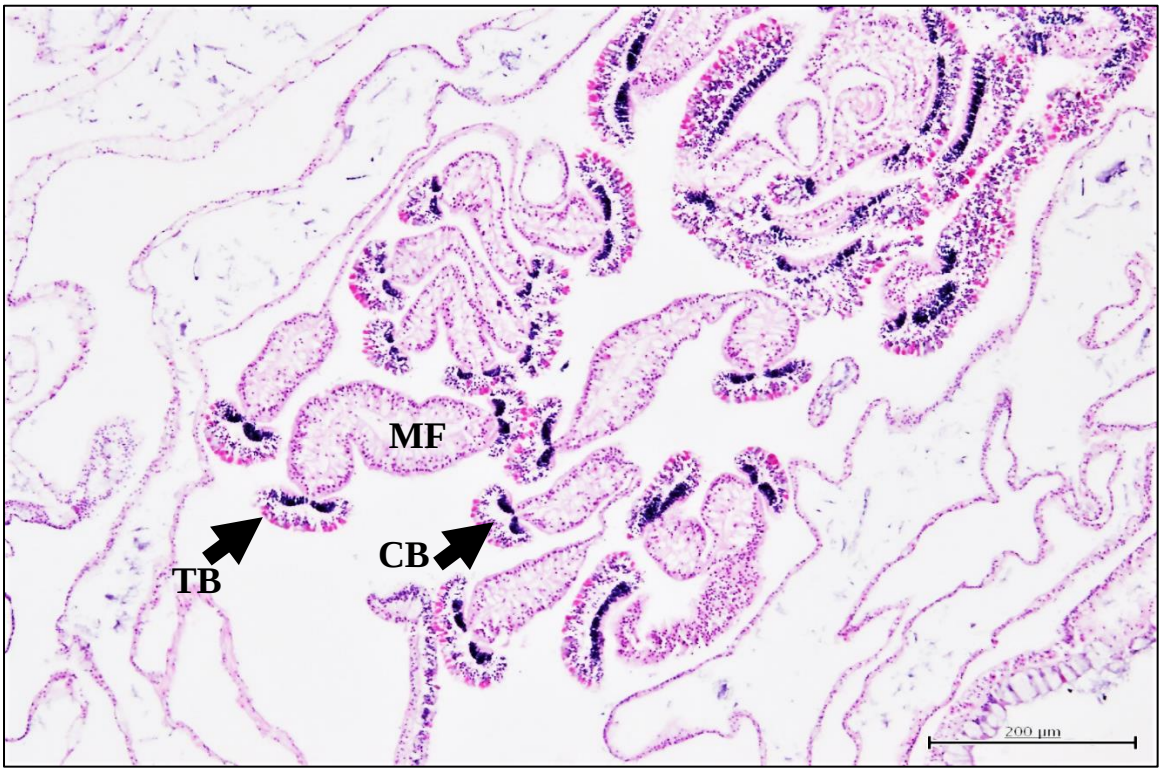


*Figure 12b. Terminal bars (**TB**) of cnidoglandular bands (**CB**) on free edge of mesenterial filaments (**MF**) have minute gaps indicating loss of ciliated cells in chemical treatment sample*

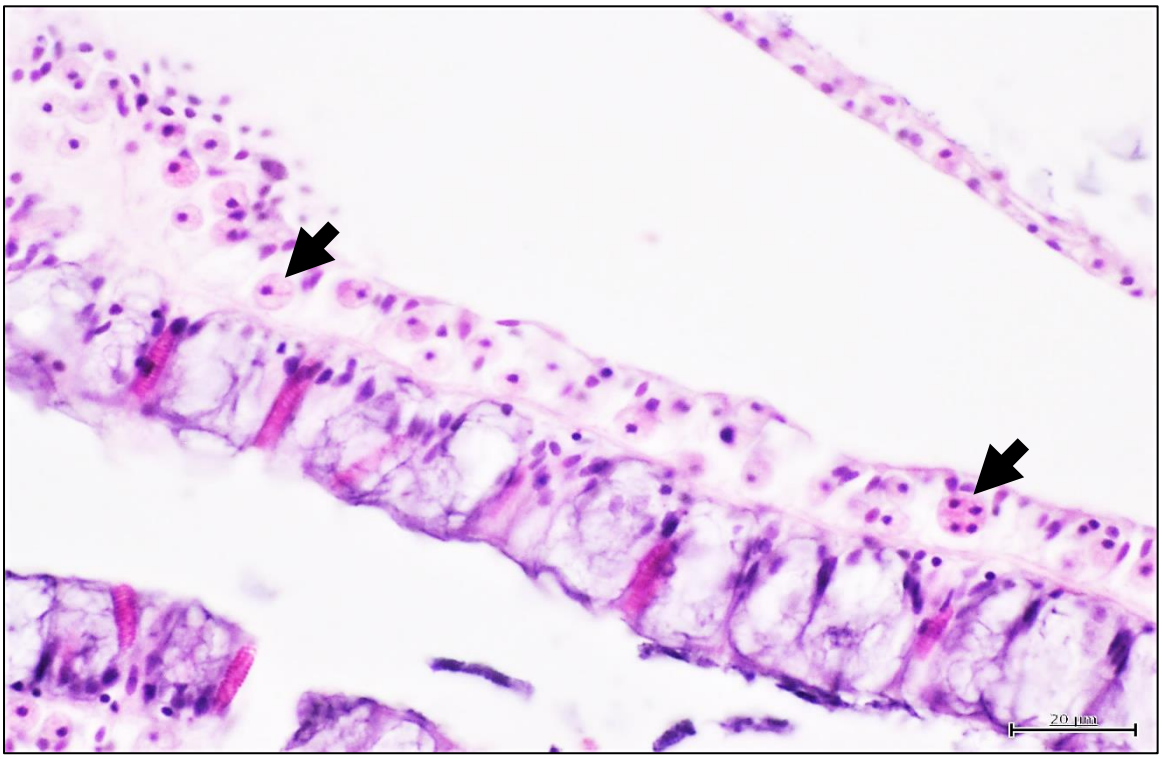


Figure 13a. Division of zooxanthellae in the surface body wall gastrodermis in control sample

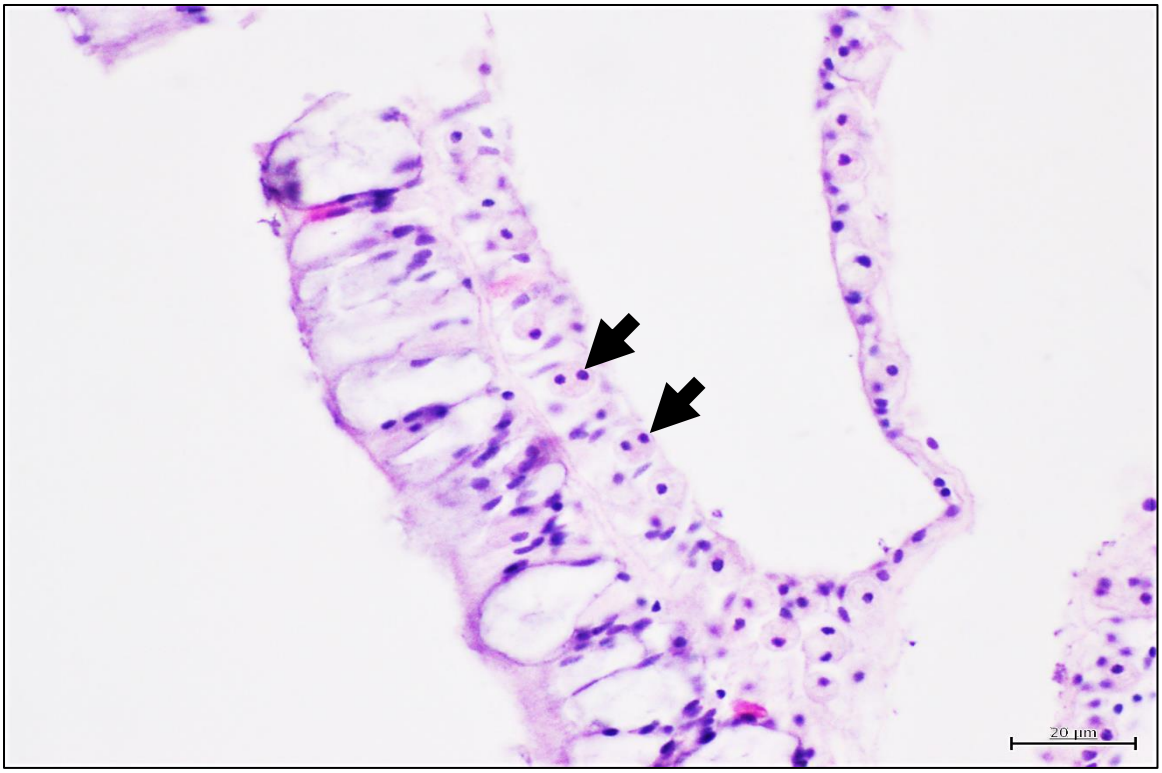


Figure 13b. Division of zooxanthellae in the surface body wall gastrodermis in mineral treatment sample

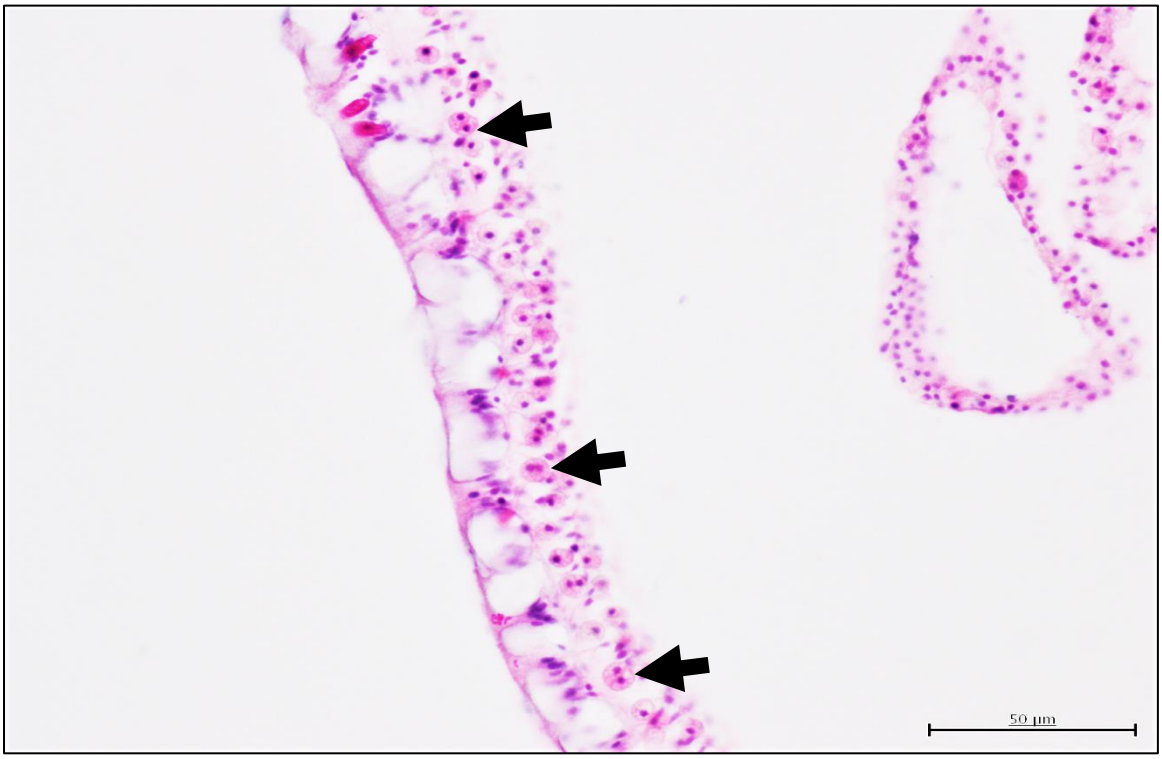


Figure 13c. Division of zooxanthellae in the surface body wall gastrodermis in chemical treatment sample

5.4 Discussion

Due to extremely low sample size (n = 2), only minimal analyses with a two-tailed t-test were performed to compare these results with other studies. The sample size was originally n = 5, but with time constraint and limited funding, only two samples were collected for each treatment and respective control. The results given must therefore be lightly considered. Although the samples were from the same genotype (K2), individual fragments responded differently, so there was variability within the genotype and both within and among sunscreen brand exposures. Despite significance of mesenterial filament RLOs (*T-test, p = 0.015*) and costal tissue loss (T-test, *p = 0.039*) in chemical treatments, error bars still overlapped when standard deviations were used for error values (Figure 7). These overlaps do not negate the significance of the means (Lanzante, 2005). During histoslide preparation, several slides were not made, because the subsamples had not been completely decalcified. Consequently, some samples had fewer subsamples that ultimately skewed the average scores for some relative condition parameters. For future research, it is recommended that a larger sample size (n = 10) be used to produce more credible results. These data were collected as a pilot study conducted by a small not-for-profit organization; it is recognized that the presented data still provides useful information for studying the toxicity of sunscreens to *A. cervicornis* and perhaps other scleractinian corals. Despite inadequate sample size, non-significant results could have also occurred due to stochasticity of trimmed sample areas, human error in scoring, exposure time and subsequent environmental conditions, and/or the phenomenon of hormesis.

With a sunscreen exposure time of one minute, corals may have only exhibited a temporary stress response. When exposed to pathogens, toxicants, sediment, or changes in environmental factors, scleractinian corals such as *A. cervicornis* may produce mucus as a sign of short-term stress (Nakajima and Tanaka, 2014). However, corals may also produce mucus during normal biological functions such as feeding and excretion of organic matter (Nakajima and Tanaka, 2014). With these observations, it could be inferred that while brief, initial contact with sunscreens may induce stress to *A. cervicornis*, it may not permanently inflict cellular damage. If exposure time was longer and samples were collected immediately following the exposure, then results would have

portrayed cellular responses of short-term stress. The benefit of collecting samples ten days after sunscreen exposure is to observe how *A. cervicornis* responds long-term to brief sunscreen exposure, and how it may affect coral growth on the reef after nursery rearing. However, resilience depends on coral health and the surrounding environmental conditions. Long-term exposure studies to observe resilience *in situ* are more difficult to achieve unless chemical pollutants are consistent and measurable within a given area. Even then, the biological phenomenon of hormesis may help corals and other organisms exposed to toxins become more resilient with time.

Hormesis is a dose-response phenomenon in which an organism experiences a positive effect from very low doses of an otherwise toxic and/or lethal substance over time (Calabrese, 2008). In this scenario, perhaps a brief exposure to small aliquots of sunscreen was enough to increase the tolerance of *A. cervicornis* to a normally- toxic substance. Hormetic responses are not completely understood and differ among species and the introduced toxicant, but it nonetheless represents a reparative process that "modestly overshoots the original homeostatic set point" (Calabrese, 2008); in other words, what does not kill you makes you stronger. Additionally, fragments' young age could have contributed to their tolerance to sunscreen exposure due to the absence of gonads in samples. To test exposure in the future, parent corals from this study could be re-exposed using the same methodologies and the results compared.

Many sunscreen manufacturers claim that their sunscreens are "reef safe", but is that true? The studies presented in this capstone clearly demonstrate that even "eco-friendly" sunscreens can have negative effects on marine organisms at very low concentrations. Some claimed "reef-safe" brands (Table 2) contain UV filters that have been shown to be toxic to marine life, both mineral and chemical (Appendix 1). The only UV filters that seem promising to the health of marine organisms are non-nano TiO_2 and non-nano ZnO, based on their larger particle size and lower solubility rates in seawater (Fabrega *et al.,* 2012; Manzo *et al.,* 2013; Spisni *et al.,* 2016). Contradicting studies, however, found that non-nano UV filters were more toxic to some marine organisms compared to smaller nanoparticles (Wong *et al.,* 2010; D'Agata *et al.,* 2014). Specifically, these studies observed DNA damage in hemocytes in filter-feeders (D'Agata *et al.,* 2014), oxidative stress in crustaceans and fish (Wong *et al.,* 2010), and

reproductive inhibition in sediment dwellers (Fabrega *et al.*, 2012) when exposed to non-nano UV filter particles. Authors from these studies indicate that these organisms may readily uptake higher concentrations of larger non-nanoparticles due to their higher bioavailability. Still, non-nano UV filters are generally lower in toxicity than other types of UV filters and seem least toxic to scleractinian corals compared to others. Unfortunately, there are no current regulations that enforce the integrity of "non-nano" and "reef-safe" advertisement claims, but consumer awareness has recently demanded that manufacturers should be more accurate (Sobek *et al.*, 2013).

6. Summary and Conclusions

UV filter compounds in commercial sunscreens have demonstrated toxic effects on marine organisms in various studies. The evolution of commercial sunscreens during the past 90 years is impressive, yet its growing industry will lead to more chemical contamination via watershed distribution pathways. Stronger global regulation of these compounds can help mitigate their release into the environment, but agreements between legislators and product companies will be a challenge. Measuring the concentrations of UV filters in marine ecosystems has proven difficult, but new toxicity models, uniform methodologies, and increased WWTP removal efficiencies are working to overcome that obstacle.

CRF's case study showed that although briefly handling *A. cervicornis* with sunscreen-laden hands (either mineral or chemical) did not seem to cause long-term damage, it could have induced stress that may lower the corals' resilience to other stressors such as environmental changes or disease. Although the number of collected fragments meant that the observations did not have enough replicates to test the hypothesis, it is hoped that these techniques and literary research can be continued and expanded for further understanding of how UV filter exposure may affect future coral restoration.

Based on the literary and histological research performed, sunscreens containing organic, chemical UV filters should be avoided completely in everyday use and while handling coral fragments within nurseries. Since all marine organisms have different cellular compositions and stress responses, no two individuals may react the same when

exposed to various types of UV filters. Additionally, environmental factors may either increase or decrease an organism's tolerance for toxicants, making it more difficult to determine effects in research. However, only non-nano TiO_2 and non-nano ZnO UV filters should be used by consumers and coral restoration groups to reduce (albeit not completely eliminate) toxicity exposure to organisms on coral reefs and beyond. Even better, wearing sun-protective clothing and reducing our sun exposure is conceivably the best option for both human health and the ocean.

7. Acknowledgements

I would like to express my sincere thanks to my capstone committee, Dr. Esther Peters and Dr. Joshua Feingold, for their professional guidance and expertise on this subject. Special thanks to Dr. Peters for handing this project to me and preparing the histoslides needed for analyses; I could not have done it without you. Therefore, my gratitude is also extended to George Mason University and the volunteers who devoted their time with Dr. Peters to prepare this data. Of course, I would like to thank the Coral Restoration Foundation for allowing me to use their case study to support my project. Ultimately, the success of this capstone would not be possible without the unwavering support of my family and loved ones. To you all, I owe everything, and it is my hope that this education will help me give back to the world what I have so thankfully been given.

8. Appendix 1

Appendix 1
Summary of Marine Toxicity Studies Using Common UV Filters

Author(s)	Date	UV Filters	Exposure Subjects	Toxicity Endpoints	Results
Danovaro *et al.*	2003	Ecamsule	Marine bacterioplankton	Viral abundance, enzymatic activities	Virus production increased; sunscreen can modify C, N, and P biogeochemical cycling in seawater
Coronado *et al.*	2008	Oxybenzone	*Paralichthys californicus*	Vitellogenin analysis	Endocrine disruption and reproduction endpoints occur only at concentrations above environmental norms
Danovaro *et al.*	2008	Octinoxate, Octocrylene, Oxybenzone, Octisalate, Avobenzone, Enzacamene	*Acropora divaricata*, *Acropora cervicornis*, *Acropora pulchra*, *Acropora aspera*, *Acropora intermedia*, *Acropora* sp., *Millepora complanata*, *Stylophora pistillata*	Zooxanthellae count, visual calorimetric analysis	Rapid/complete coral bleaching at 10 µL/L within 96 hours; response not dose-dependent; sunscreens promoted viral infections
Canesi *et al.*	2010 a	Nano titanium dioxide	*Mytilus galloprovincialis*	Hemocyte condition, immune parameters, ROS production, MAPK signaling	NP suspensions did not significantly affect lysosomal membrane stability, but dose-dependent lysozyme release and inflammatory effects observed
Canesi *et al.*	2010 b	Nano titanium dioxide	*Mytilus galloprovincialis*	Lysosomal oxidative stress parameters, gill antioxidant	ROS production, digestive stress, lysosomal oxidative stress,

Author(s)	Date	UV Filters	Exposure Subjects	Toxicity Endpoints	Results
				enzyme activities	and gill antioxidant enzyme activities occurred.
Fent *et al.**	2010	Enzacamene, Octinoxate, Oxybenzone, Sulisobenzone 3-benzylidene camphor	*Daphnia magna*	48-hour acute immobilization assay (OECD Guideline 202)	No adverse effects observed; may only pose risk for sensitive aquatic organisms
Galloway *et al.*	2010	Nano titanium dioxide ($nTiO_2$)	*Arenicola marina*	Gut histology, comet assay (DNA damage)	Dose-dependent adverse effects on feeding; DNA and cell damage
Miao *et al.*	2010	Nano zinc oxide (nZnO)	*Thalassiosira pseudonana*	Cell-specific growth rate μ, cellular chlorophyll *a* production	Inhibitive effects mainly caused by Zn^{2+} ions but not nZnO
Miller *et al.*	2010	Nano zinc oxide (nZnO), Nano titanium dioxide ($nTiO_2$)	*Isochrysus galbana*, *Thalassiosira pseudonana*, *Dunaliella tertiolecta*, *Skeletonema marinoi*	Population growth rate	$nTiO_2$ had no effect on growth rates, whereas nZnO significantly depressed growth rates of all species; ZnO toxicity likely due to Zn^{2+} ions.
Wong *et al.*	2010	Nano zinc oxide (nZnO), Non-nano zinc oxide (Non-nano ZnO)	*Skeletonema costatum*, *Thalassiosia pseudonana*, *Tigriopus japonicus*, *Elasmopus rapax*, *Oryzias melastigma**	Growth rate, mortality, protein quantification, ion solubility, oxidative stress biomarkers	nZnO is more toxic to algae due to Zn^{2+} charge; non-nano ZnO more toxic to crustaceans and fish due to higher bioavailability
Kusk *et al.*	2011	Benzophenone -1 (BP-1)	*Acartia tonsa*	Mortality, growth rate	BP-1 acutely toxic at 2.6 mg/L but varied with environmental conditions
Miglietta *et al.*	2011	Nano zinc oxide (nZnO)	*Paracentrotus lividus*, *Artemia salina*,	Embryotoxicity, acute toxicity, growth inhibition	Growth inhibition observed in all algae; *D. tertiolecta* most

Author(s)	Date	UV Filters	Exposure Subjects	Toxicity Endpoints	Results
			Dunaliella tertiolecta, *Isocrysis galbana*, *Tetraselmis suecica*		sensitive alga to nZnO; centrifugation lowers toxic effect overall; *P. lividus* most sensitive overall to nZnO
Peng *et al.*	2011	Nano zinc oxide	*Thalassiosira pseudonana*, *Chaetoceros gracilis*, *Phaeodactylum tricornutum*	Cell count, Log-linear cell division rate	Inhibited growth of *T. pseudonana* and *C. gracilis* at all concentrations; *P. tricornutum* was least sensitive
Zhu *et al.*	2011	Nano titanium dioxide ($nTiO_2$)	*Haliotis diversicolor supertexta*	Spectrophotometry, enzymatic activity, biochemical assays	Oxidative stress, though $nTiO_2$ not acutely toxic
Buffet *et al.*	2012	Nano zinc oxide (nZnO)	*Scrobicularia plana*, *Hediste diversicolor*	Isotope tracing, biochemical markers, burrowing activity	Impaired burrowing behavior and feeding rate in both species; no adverse effects at environmental concentrations
Fabrega *et al.*	2012	Nano zinc oxide, Non-nano zinc oxide, Zn^{2+} ions	*Corophium volutator*	Mortality, growth, and reproductive rate	Growth and reproductive inhibition observed for all zinc forms.
Miller *et al.*	2012	Nano titanium dioxide	*Isochrysus galbana*, *Thalassiosira pseudonana*, *Dunaliella tertiolecta*, *Skeletonema costatum*	Cell density	Increased ROS production in seawater, increased oxidative stress, and decreased resiliency
Skelly *et al.*	2012	Banana Boat SPF 50: Avobenzone, Homosalate, Octocrylene,	*Pocillopora* spp.	Visual color scale	Bleaching occurred, but concentration insignificant; mere exposure caused bleaching.

Author(s)	Date	UV Filters	Exposure Subjects	Toxicity Endpoints	Results
		Octisalate, Oxybenzone			
Barmo *et al.*	2013	Nano titanium dioxide	*Mytilus galloprovincialis*	Lysosomal membrane stability, hemocyte analysis	Lysosomal membrane destabilization; changes in oxidative stress biomarkers.
Jarvis *et al.*	2013	Nano zinc oxide (nZnO)	*Acartia tonsa* (exposed to nZnO through phytoplankton diet of *Thalassiosira weissflogii*)	Growth rate	Dose-dependent growth reduction of *T. weissflogii*; decreased *A. tonsa* survival and reproduction.
Libralato *et al.*	2013	Nano titanium dioxide	*Mytilus galloprovincialis*	Retarded or malformed larvae count	Malformed larvae after first metamorphosis from trochophore stage
Manzo *et al.*	2013	Nano zinc oxide (nZnO), Non-nano zinc oxide (non-nZnO)	*Dunaliella tertiolecta*	Growth rate, concentration-response functions	nZnO more toxic than non-nZnO by growth rate inhibition; toxicity is particle-size dependent.
Tovar-Sánchez *et al.*	2013	Various organic and inorganic UV filter formulas, unspecified	*Chaetoceros gracilis*	Growth rate	Average EC_{50} = 125±71 mg L^{-1} (> environmental samples); growth rate inhibition; spray sunscreens demonstrated highest toxicity
Castro-Bugallo *et al.*	2014	Nano zinc oxide	*Phaedodactylum tricornutum*, *Alexandrium minutum*, *Tetraselmis suecica*	Growth assays, ROS detection, microalgal cell autofluorescence, cell carbon and nitrogen analysis, intracellular metal analysis	*P. tricornutum* and *A. minutum* exhibited decreased chlorophyll fluorescence and high ROS, but not *T. suecica*
Clemente *et al.*	2014	Nano titanium dioxide ($nTiO_2$)	*Artemia salina*	Growth rate, oxidative stress and metabolism biomarkers	UV light enhanced toxicity ($EC50_{48h}$ = 4 mg/L); adverse

Author(s)	Date	UV Filters	Exposure Subjects	Toxicity Endpoints	Results
					effects dependent on organism, exposure time, $nTiO_2$ crystal phase, and light condition.
D'Agata *et al.*	2014	Non-nano titanium dioxide (non-$nTiO_2$), Nano titanium dioxide ($nTiO_2$)	*Mytilus galloprovincialis*	Hemolymph analysis, Comet assay, acid mucocyte quantification, metal oxide concentration in tissue samples	$nTiO_2$ accumulation higher, but non-$nTiO_2$ may be more toxic; DNA damage to hemocytes; photocatalytic aging does not significantly alter $nTiO_2$ toxicity
Downs *et al.*	2014	Benzophenone -2 (BP-2)	*Stylophora pistillata*	Chlorophyll fluorescence, DNA abasic lesions, tissue and cellular pathomorphology assessment	Increased bleaching in response to increasing BP-2 concentrations; BP-2 transformed planulae from motile to sessile and deformed.
Jovanović and Guzmán	2014	Nano titanium dioxide ($nTiO_2$)	*Orbicella faveolata*	Zooxanthellae count, mass spectrometry	Zooxanthellae expulsion; $nTiO_2$ bioaccumulation in microflora
Paredes *et al.*	2014	Enzacamene (4-MBC), Octinoxate (EHMC), Oxybenzone (BP-3), Sulisobenzone (BP-4)	*Isochrysis galbana*, *Mytilus galloprovincialis*, *Paracentrotus lividus*, *Siriella armata*	Growth rate, larval abnormality, larval size, mortality, cell count	EHMC and 4-MBC most toxic for test species, followed by BP-3 and BP-4; microalgae was most affected. Measured water samples 10–100 s ng L^{-1}
Petersen *et al.*	2014	Oxybenzone (BP-3)	*Skeletonema pseudocostatum*	Growth rate	BP-3 was fourth least toxic of 10 other non-UV filter tested compounds (EC_{50} = 1.1 μM)

Author(s)	Date	UV Filters	Exposure Subjects	Toxicity Endpoints	Results
Sánchez-Quiles and Tovar-Sánchez	2014	Nano titanium dioxide, Nano zinc oxide, Oxybenzone, Octocrylene, Octinoxate, *p*-aminobenzoic acid (PABA), Ensulizole	Marine phytoplankton (*unspecified*)	Cellular chlorophyll *a* production	H_2O_2 production from inorganics by photoexcitation under UV radiation causes cellular stress in marine phytoplankton, but organics may also contribute
Aravantinou *et al.*	2015	nZnO	*Dunaliella tertiolecta*, *Tetraselmis suecica*	Growth rate	*D. tertiolecta* and *T. suesica* more sensitive than freshwater species; IC_{50} < 2.57 mg/L)
Sharp *et al.*	2015	Oxybenzone, Non-nano titanium dioxide (Non-nTiO_2)	*Porites astreoides*	Mortality, settlement assays	Non-nTiO_2: no significant pre-settlement larval mortality or reduction in larval settlement BP-3: larval settlement inhibition; no significant pre-settlement mortality
Suman *et al.*	2015	Nano zinc oxide (nZnO)	*Chlorella vulgaris*	Cell viability, lactate dehydrogenase assay, oxidative stress	Cytotoxic effects observed; significant oxidative stress; decreased cell viability
Xia *et al.*	2015	Nano titanium dioxide (nTiO_2)	*Nitzschia closterium*	Growth rate, enzymatic activity, lipid peroxidation, ROS production	Induced algal cell membrane damage; nanotoxicity caused by ROS levels from internalization of TiO_2 nanoparticles
Yung *et al.*	2015	Nano zinc oxide (nZnO)	*Thalassiosira pseudonana*	Growth rate, chlorophyll fluorescence	Decreased toxicity with increased salinity; toxicity

Author(s)	Date	UV Filters	Exposure Subjects	Toxicity Endpoints	Results
					partly due to dissolved Zn^{2+}
Downs *et al.*	2016	Oxybenzone	*Stylophora pistillata*	Chlorophyll fluorescence, DNA abasic lesions, tissue and cellular pathomorphology assessment	Planulae exhibited an increasing rate of coral bleaching in response to increasing concentrations of oxybenzone; BP-3 transformed planulae from motile to sessile and deformed.
Hazeem, *et al.*	2016	Nano zinc oxide, Nano titanium dioxide	*Picochlorum* sp.	Growth rate, chlorophyll *a* concentration	Inhibited algal growth and chlorophyll *a* concentration during early growth stages; no significant effects during late growth stages
McCoshum *et al.*	2016	Equate brand: Homosalate, Oxybenzone, Octocrylene, Octisalate, Avobenzone	*Convolutriloba macropyga*, *Nitzschia* sp., *Aiptasia* sp., *Xenia* sp.	Population/colony growth, behavioral analyses	Exposed flatworms and pulse corals had reduced population and colony growth and abnormal behavior; *Aiptasia* were categorized unhealthy, and *Nitzschia* had reduced biomass and fluorescence
Shiavo *et al.*	2016	Nano zinc oxide (nZnO), Nano titanium dioxide ($nTiO_2$)	*Dunaliella tertiolecta*	Cell division inhibition, growth inhibition	nZnO particles act firstly in cell division inhibition; nZnO toxicity mainly Zn^{2+} ion release; $nTiO_2$ more toxic than nZnO
Spisni *et al.*	2016	Nano zinc oxide (industrial &	*Thalassiosira pseudonana*	Growth inhibition	Industrial more toxic than commercial due

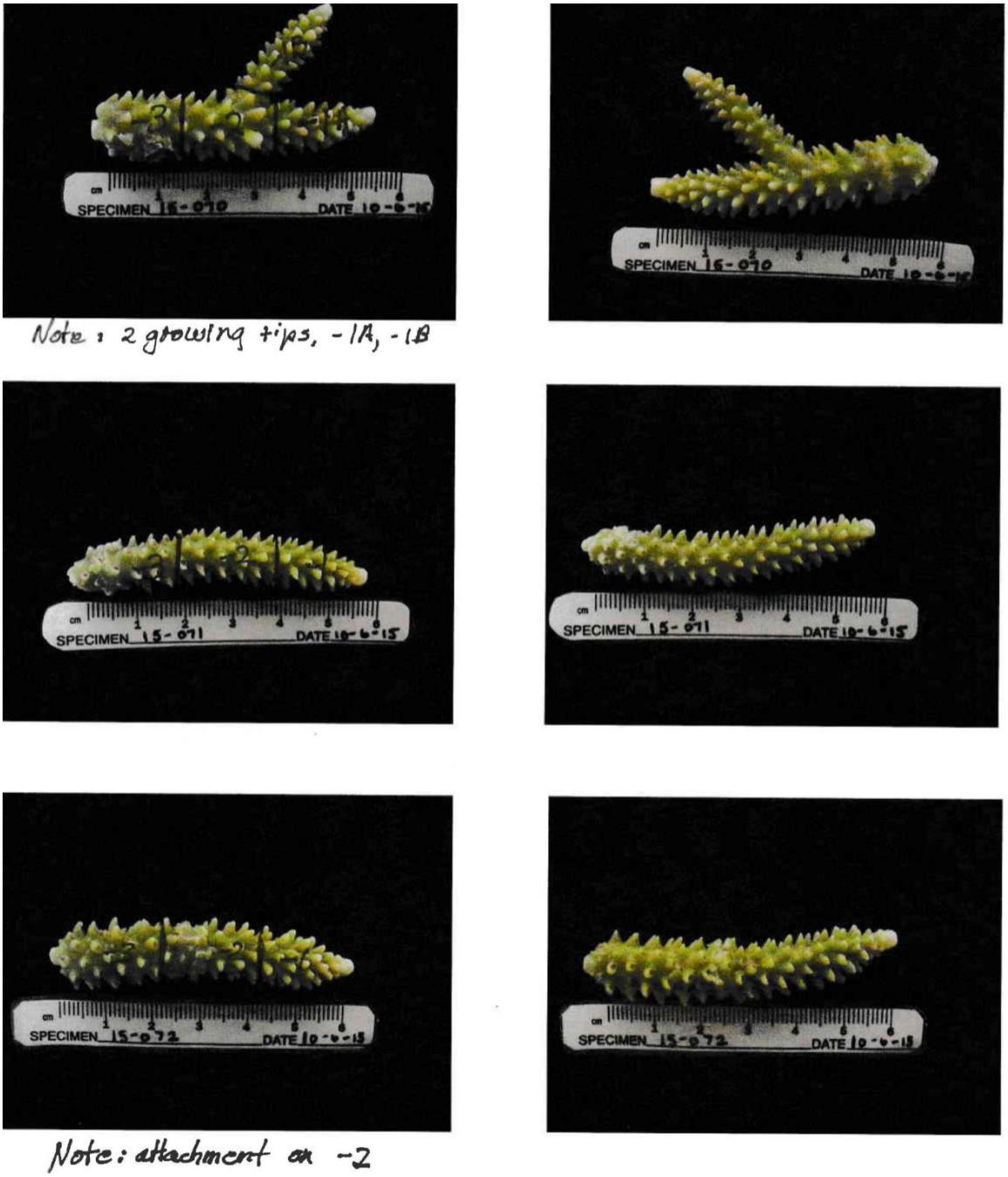
Author(s)	Date	UV Filters	Exposure Subjects	Toxicity Endpoints	Results
		commercial types)			to particle size; growth inhibition increased with exposure time.
Wang *et al.*	2016	Nano titanium dioxide ($nTiO_2$)	*Phaeodactylum tricornutum*	Growth inhibition, photosynthetic pigment content determination, cell integrity analysis	$nTiO_2 \geq 20$ mg/L could significantly inhibit *P. tricornutum* growth; oxidative stress observed
Zhang *et al.*	2016	Nano zinc oxide (nZnO), Non-nano zinc oxide(Non-nZnO)	*Skeletonema costatum*	Growth inhibition, lipid peroxidation injury, Zn^{2+} ion accumulation	nZnO more toxic than non-nZnO; higher Zn^{2+} ion uptake under nZnO treatment than non-nZnO

**- denotes freshwater exposure study, included for results comparison*

9. Appendix 2

Appendix 2

Sample Trim Sheet of *A. cervicornis* Fragments



10. Appendix 3

Appendix 3

Scoring Rubric for Histopathological Analyses of *A. cervicornis*
(Adopted from Miller *et al*. 2014, developed by Dr. Esther Peters)

Parameter Viewed at 100x or 250+x, Description of "Normal"	**Numerical Score Intensity or Severity Score**				
0 (No Change)	**1 (Very Good)**	**2 (Good)**	**3 (Fair)**	**4 (Poor)**	**5 (Very Poor)**
General Condition 0 = Excellent, similar to 1970s samples, thick epithelia and mesoglea, mucocytes not hypertrophied, highly cellular	Similar to 1970s samples, but epithelia and mesoglea not as thick, epidermal mucocytes slightly hypertrophied	Hypertrophy of epidermal mucocytes, intact epithelia and mesoglea, mesentery and filament architecture still normal	Hypertrophy of epidermal mucocytes, minimal to mild attenuation (atrophy) of epithelia and mesoglea noted	Loss of mucocytes, moderate attenuation of epithelia and mesoglea, mesentery and filament architecture degenerating	Severe attenuation of epithelia and mesoglea, loss of epitheliomuscular cells with vacuolation of mesogleal pleats necrosis and dissociation of mesenterial filaments, necrosis and lysing of epithelial cells
Zooxanthellae 0 = Gastrodermal cells packed with well-stained algal symbionts in surface body wall, tentacles; scattered algal symbionts deeper in gastrovascular canals and absorptive cells next to mesenterial filaments	Similar to 1970's samples, thick layer of well-stained algal symbionts in gastrodermis of surface body wall, tentacles, and scattered cells in gastrovascular canals and absorptive cells next to mesenterial filaments	Thick layer of well-stained algal symbionts, but not quite as abundant as in 1970's samples	Algal symbionts fewer in gastrodermis which is mildly attenuated (atrophied), most still stain appropriately	Single row of algal symbionts in surface body wall gastrodermis and markedly fewer in tentacle gastrodermis, some have lost acidophilic staining as proteins no longer produced or nucleus/cytoplasm lysed, vacuole enlarged compared to algal cell	No zooxanthellae present in cuboidal gastrodermal cells of colony (bleached)
Epidermal Mucocytes 0 = In 1970s sample, thin columnar cells, uniform distribution and not taller than ciliated supporting cells, pale mucus	Slightly hypertrophied, numerous, pale-staining frothy mucus	Many cells hypertrophied, abundant release of pale-staining mucus	Uneven appearance of mucocytes, some hypertrophied but some reduced in size and secretion, darker staining mucus	Some epidermal foci lack mucocytes entirely, attenuation (atrophy) of epidermis evident, darker staining and stringy mucus	Loss of many mucocytes, epidermis is attenuated to at least half of normal thickness or more, if mucus present, it stains dark, thick
Cnidoglandular Band Epithelium Mucocytes	Less than half the area of cnidoglandular band is mucocytes, but	About half the area is mucocytes, some hypertrophied	About half the area is mucocytes, all hypertrophied	About three quarters of the area is mucocytes, mucus production reduced, some	Loss of mucocytes, vacuolation and necrosis of cells present

Parameter Viewed at 100x or 250+x, Description of "Normal"	**Numerical Score Intensity or Severity Score**				
0 (No Change)	**1 (Very Good)**	**2 (Good)**	**3 (Fair)**	**4 (Poor)**	**5 (Very Poor)**
0 = Oral portion lacks mucocytes, increasing in number aborally, may be abundant with pale mucus; difficult to assess significance of appearance	could be more depending on location along the filament, size of mucocytes variable			vacuolation present	
Degeneration of Cnidoglandular Bands 0 = Ciliated columnar cells, nematocytes, acidophilic granular gland cells, and mucocytes abundant (but varying with location), tall, thin columnar, contiguous, terminal bar well formed	Mild reduction in cell height	Cell height more reduced, mild loss of mucocytes or secretions	Attenuation (atrophy), loss of cells	Moderate attenuation of epithelium, some granular gland cells stain dark pink and are rounded, not columnar, terminal bar not contiguous, some pycnotic nuclei present, loss of cells by detachment and sloughing	Severe atrophy of epithelium, detachment from mesoglea and loss of cells, necrosis or apoptosis of remaining cells, no terminal bar present, loss of cilia
Dissociation of Cells on Mesenterial Filaments 0 = All cells intact and within normal limits, contiguous, thin columnar morphology, terminal bar present, cilia visible along apical surface	Minimal loss of cilia, but will not be present where mucocytes are predominant	Minimal to mild loss of cells, terminal bar has minute gaps indicating loss of ciliated cells	Attenuation (atrophy) of cells, vacuolation, reduced cilia, but filament still intact	Rounding up and loss of granular gland cells, some pycnotic nuclei present, cell loss evident, terminal bar gaps, terminal web (junctions) between cells lost, starting to spread apart along cnidoglandular band	Marked to severe separation of cells, most necrotic with pycnotic nuclei, vacuolated, lysing and loss of mucocytes, nematocysts, granular gland cells and ciliate columnar cells
Costal Tissue Loss 0 = Tissue covering costae intact, epidermis similar in thickness to epidermis of surface body wall with gastrodermis as it covers the costae, although this may vary with location and be thinner;	Attenuation (atrophy) of epidermis, mesoglea, and calicodermis, but still intact over costae	Up to one-quarter of costae on corallite surfaces exposed due to loss of epithelia and mesoglea	Up to one-half of costae exposed	About three quarters of costae exposed	Most costae exposed or gaps in surface body wall, tissues atrophied

Parameter Viewed at 100x or 250+x, Description of "Normal"	**Numerical Score Intensity or Severity Score**				
0 (No Change)	**1 (Very Good)**	**2 (Good)**	**3 (Fair)**	**4 (Poor)**	**5 (Very Poor)**
calicodermis thick, pale to clear cytoplasm, or thinner with cytoplasmic extensions apically					
Calicodermis Condition 0 = Calicoblasts numerous, squamous but thick cytoplasm	Calicoblasts slightly reduced in height focally (more likely interior of colony)	About half of calicoblasts attenuated (atrophied), loss of proteins in cytoplasm	Most calicoblasts attenuated, fewer in number, spread out thinly on mesoglea, still cuboidal to columnar and active under surface body wall and in apical polyps	Most calicoblasts markedly atrophied, fewer in number, some separating from mesoglea	Surface body wall calicoblasts severely atrophied or vacuolated, detaching and sloughing, missing from mesoglea
Epidermal RLOs 0 = Not present	One infected cell on oral disks or tentacles of polyps (rare)	Several infected cells on oral disks or tentacles of polyps, numerous mucocytes present (occasional)	About half of mucocytes infected on oral disks or tentacles of polyps, loss of some mucocytes (common), rare infected cells in actinopharynx epidermis	More than half of mucocytes infected on oral disks or tentacles of polyps, loss of mucocytes (frequent), increase in infected cells on actinopharynx epidermis	Nearly all remaining mucocytes infected (may have lost many as infected cells die and lyse), many infected cells in actinopharynx epidermis (abundant)
Filament RLOs 0 = Not present	One infected cell on cnidoglandular bands (rare)	Several infected cells on cnidoglandular bands present in tissue section (occasional)	Infected cells present on about half of sections through cnidoglandular bands (common), slight loss of mucocytes, a few infected mucocytes in gastrodermis lining gastrovascular canals (rare)	A few infected cells present on almost all sections through cnidoglandular bands (frequent), loss of mucocytes, more infected cells in gastrodermis lining gastrovascular canals (common)	Nearly all remaining mucocytes infected but many lost as infected cells die and lyse, mucocytes of gastrodermis or mesenteries infected (abundant)

11. Literature Cited

Abid, Abdul Rahman, Bronisław Marciniak, Tomasz Pędziński, and Muhammad Shahid. "Photo-Stability and Photo-Sensitizing Characterization of Selected Sunscreens' Ingredients." *Journal of Photochemistry and Photobiology A: Chemistry* 332 (2017): 241-50.

"*Acropora cervicornis*". The IUCN Red List of Threatened Species. http://www.iucnredlist.org/details/133381/0 (accessed March 17, 2018).

Alloway, Brian, and David C Ayres. *Chemical Principles of Environmental Pollution*. CRC Press. (1997).

Aravantinou, Andriana F, Vasiliki Tsarpali, Stefanos Dailianis, and Ioannis D Manariotis. "Effect of Cultivation Media on the Toxicity of ZnO Nanoparticles to Freshwater and Marine Microalgae." *Ecotoxicology and Environmental Safety* 114 (2015): 109-16.

Axelstad, Marta, Ulla Hass, Karin Lund Kinnberg, and Poul Bjerregaard. "Assessment of the Endocrine Disrupting Potential of 23 UV-Filters" (J. No. Mst-656-00150). (2013).

Bachelot, Morgane, Zhi Li, Dominique Munaron, Patrik Le Gall, Claude Casellas, Hélène Fenet, and Elena Gomez. "Organic UV Filter Concentrations in Marine Mussels from French Coastal Regions." *Science of the Total Environment* 420 (2012): 273-79.

Baker, Tony J, Charles R Tyler, and Tamara S Galloway. "Impacts of Metal and Metal Oxide Nanoparticles on Marine Organisms." *Environmental Pollution* 186 (2014): 257-71.

Barmo, Cristina, Caterina Ciacci, Barbara Canonico, Rita Fabbri, Katia Cortese, Teresa Balbi, Antonio Marcomini, *et al.* "In Vivo Effects of N-TiO2 on Digestive Gland and Immune Function of the Marine Bivalve *Mytilus galloprovincialis*." *Aquatic Toxicology* 132 (2013): 9-18.

Barnard, Amanda S, Jordan J Louviere, Edward Wei, and Leon Zadorin. "Using Hypothetical Product Configurators to Measure Consumer Preferences for Nanoparticle Size and Concentration in Sunscreens." *Design Science* 2 (2016).

Boch, Charles A, and Aileen NC Morse. "Testing the Effectiveness of Direct Propagation Techniques for Coral Restoration of Acropora Spp." *Ecological Engineering* 40 (2012): 11-17.

Bondarenko, Olesja, Katre Juganson, Angela Ivask, Kaja Kasemets, Monika Mortimer, and Anne Kahru. "Toxicity of Ag, CuO and ZnO Nanoparticles to Selected

Environmentally Relevant Test Organisms and Mammalian Cells in Vitro: A Critical Review." *Archives of Toxicology* 87, no. 7 (2013): 1181-200.

Brausch, John M., and Gary M. Rand. "A Review of Personal Care Products in the Aquatic Environment: Environmental Concentrations and Toxicity." *Chemosphere* 82, no. 11 (2011/03/01/ 2011): 1518-32.

Buffet, Pierre-Emmanuel, Claude Amiard-Triquet, Agnieszka Dybowska, Christine Risso-de Faverney, Marielle Guibbolini, Eugenia Valsami-Jones, and Catherine Mouneyrac. "Fate of Isotopically Labeled Zinc Oxide Nanoparticles in Sediment and Effects on Two Endobenthic Species, the Clam *Scrobicularia plana* and the Ragworm *Hediste diversicolor*." *Ecotoxicology and Environmental Safety* 84 (2012): 191-98.

Calabrese, Edward J. "Hormesis: Why It Is Important to Toxicology and Toxicologists." *Environmental Toxicology and Chemistry* 27, no. 7 (2008): 1451-74.

Canesi, Laura, Caterina Ciacci, Davide Vallotto, Gabriella Gallo, Antonio Marcomini, and Giulio Pojana. "In Vitro Effects of Suspensions of Selected Nanoparticles (C60 Fullerene, TiO2, SiO2) on Mytilus hemocytes." *Aquatic Toxicology* 96, no. 2 (2010a): 151-158.

Canesi, L., R. Fabbri, G. Gallo, D. Vallotto, A. Marcomini, and G. Pojana. "Biomarkers in *Mytilus galloprovincialis* Exposed to Suspensions of Selected Nanoparticles (Nano Carbon Black, C60 Fellerene, Nano-TiO2, Nano-SiO2)." *Aquatic Toxicology* 100, no. 2 (2010b): 168-177.

Carp, Oana, Carolien L Huisman, and Armin Reller. "Photoinduced Reactivity of Titanium Dioxide." *Progress inSsolid State Chemistry* 32, no. 1 (2004): 33-177.

Castro-Bugallo, Alexandra, África González-Fernández, Cástor Guisande, and Aldo Barreiro. "Comparative Responses to Metal Oxide Nanoparticles in Marine Phytoplankton." *Archives of Environmental Contamination and Toxicology* 67, no. 4 (2014): 483-93.

Chapman, Peter M. "Determining When Contamination Is Pollution—Weight of Evidence Determinations for Sediments and Effluents." *Environment International* 33, no. 4 (2007): 492-501.

Chapman, Peter M, James Downie, Allan Maynard, and Laura A Taylor. "Coal and Deodorizer Residues in Marine Sediments—Contaminants or Pollutants?". *Environmental Toxicology and Chemistry* 15, no. 5 (1996): 638-42.

Chen, Feiran, Christian Huber, and Peter Schröder. "Fate of the Sunscreen Compound Oxybenzone in Cyperus Alternifolius Based Hydroponic Culture: Uptake, Biotransformation and Phytotoxicity." *Chemosphere* 182 (2017): 638-46.

Clemente, Z, VL Castro, CM Jonsson, and LF Fraceto. “Minimal Levels of Ultraviolet Light Enhance the Toxicity of TiO2 Nanoparticles to Two Representative Organisms of Aquatic Systems.” *Journal of Nanoparticle Research* 16, no. 8 (2014): 2559.

Coral Restoration Foundation. “Restoring and Protecting Reefs.” Coralrestoration.org (accessed March 17, 2018).

Coronado, Michael, Hector De Haro, Xin Deng, Mary Ann Rempel, Ramon Lavado, and Daniel Schlenk. “Estrogenic Activity and Reproductive Effects of the UV-Filter Oxybenzone (2-Hydroxy-4-Methoxyphenyl-Methanone) in Fish.” *Aquatic Toxicology* 90, no. 3 (2008): 182-87.

D'Agata, Alessia, Salvatore Fasulo, Lorna J Dallas, Andrew S Fisher, Maria Maisano, James W Readman, and Awadhesh N Jha. “Enhanced Toxicity of ‘Bulk' Titanium Dioxide Compared to ‘Fresh'and ‘Aged' Nano-TiO2 in Marine Mussels (*Mytilus galloprovincialis*).” *Nanotoxicology* 8, no. 5 (2014): 549-58.

Danovaro, R, and C Corinaldesi. “Sunscreen Products Increase Virus Production through Prophage Induction in Marine Bacterioplankton.” *Microbial Ecology* 45, no. 2 (2003): 109-18.

Danovaro, Roberto, Lucia Bongiorni, Cinzia Corinaldesi, Donato Giovannelli, Elisabetta Damiani, Paola Astolfi, Lucedio Greci, and Antonio Pusceddu. “Sunscreens Cause Coral Bleaching by Promoting Viral Infections.” *Environmental Health Perspectives* 116, no. 4 (2008): 441.

Díaz-Cruz, M Silvia, Marta Llorca, and Damià Barceló. “Organic UV Filters and Their Photodegradates, Metabolites and Disinfection by-Products in the Aquatic Environment.” *TrAC Trends in Analytical Chemistry* 27, no. 10 (2008): 873-87.

Díaz-Cruz, M Silvia, and Damià Barceló. *Personal Care Products in the Aquatic Environment*. Vol. 36: Springer, 2015.

Downs, CA, Esti Kramarsky-Winter, John E Fauth, Roee Segal, Omri Bronstein, Rina Jeger, Yona Lichtenfeld, *et al.* “Toxicological Effects of the Sunscreen UV Filter, Benzophenone-2, on Planulae and in Vitro Cells of the Coral, *Stylophora pistillata*.” *Ecotoxicology* 23, no. 2 (2014): 175-91.

Downs, CA, Esti Kramarsky-Winter, Roee Segal, John Fauth, Sean Knutson, Omri Bronstein, Frederic R Ciner, *et al.* “Toxicopathological Effects of the Sunscreen UV Filter, Oxybenzone (Benzophenone-3), on Coral Planulae and Cultured Primary Cells and its Environmental Contamination in Hawaii and the US Virgin Islands.” *Archives of Environmental Contamination and Toxicology* 70, no. 2 (2016): 265-88.

Dunphy Guzman, Katherine A, Michael P Finnegan, and Jillian F Banfield. "Influence of Surface Potential on Aggregation and Transport of Titania Nanoparticles." *Environmental Science & Technology* 40, no. 24 (2006): 7688-93.

Fabrega, Julia, Ratna Tantra, Aisha Amer, Bjorn Stolpe, Jordan Tomkins, Tony Fry, Jamie R Lead, Charles R Tyler, and Tamara S Galloway. "Sequestration of Zinc from Zinc Oxide Nanoparticles and Life Cycle Effects in the Sediment Dweller Amphipod *Corophium volutator*." *Environmental Science & Technology* 46, no. 2 (2012): 1128-35.

Fent, Karl, Petra Y Kunz, Armin Zenker, and Maja Rapp. "A Tentative Environmental Risk Assessment of the UV-Filters 3-(4-Methylbenzylidene-Camphor), 2-Ethyl-Hexyl-4-Trimethoxycinnamate, Benzophenone-3, Benzophenone-4 and 3-Benzylidene Camphor." *Marine Environmental Research* 69 (2010): S4-S6.

Gago-Ferrero, Pablo, Mariana B Alonso, Carolina P Bertozzi, Juliana Marigo, Lupércio Barbosa, Marta Cremer, Eduardo R Secchi, *et al.* "First Determination of UV Filters in Marine Mammals. Octocrylene Levels in Franciscana Dolphins." *Environmental Science & Technology* 47, no. 11 (2013): 5619-25.

Gago-Ferrero, Pablo, M Silvia Díaz-Cruz, and Damià Barceló. "An Overview of UV-Absorbing Compounds (Organic UV Filters) in Aquatic Biota." *Analytical and Bioanalytical Chemistry* 404, no. 9 (2012): 2597-610.

Gallagher, CH, GE Greenoak, Vivienne E Reeve, PJ Canfield, RSU Baker, and AM Bonin. "Ultraviolet Carcinogenesis in the Hairless Mouse Skin Influence of the Sunscreen 2-Ethylhexyl-P-Methoxycinnamate." *Australian Journal of Experimental Biology & Medical Science* 62, no. 5 (1984).

Galloway, Tamara, Ceri Lewis, Ida Dolciotti, Blair D Johnston, Julian Moger, and Francesco Regoli. "Sublethal Toxicity of Nano-Titanium Dioxide and Carbon Nanotubes in a Sediment Dwelling Marine Polychaete." *Environmental Pollution* 158, no. 5 (2010): 1748-55.

Gasparro, FP. "Paba: Friend or Foe?". *Photo-dermatology* 3, no. 2 (1986): 61.

Ge, Yuan, Joshua P Schimel, and Patricia A Holden. "Evidence for Negative Effects of TiO2 and ZnO Nanoparticles on Soil Bacterial Communities." *Environmental Science & Technology* 45, no. 4 (2011): 1659-64.

Giokas, Dimosthenis L, Amparo Salvador, and Alberto Chisvert. "UV Filters: From Sunscreens to Human Body and the Environment." *TrAC Trends in Analytical Chemistry* 26, no. 5 (2007): 360-74.

Goksøyr, Anders, Knut Erik Tollefsen, Merete Grung, Katharina Løken, Elisabeth Lie, Armin Zenker, Karl Fent, Martin Schlabach, and Sandra Huber. "Balsa Raft

Crossing the Pacific Finds Low Contaminant Levels." *Environmental Science & Technology* 43, no. 13 (2009): 4783-90.

Hazeem, Layla J, Mohammed Bououdina, Suad Rashdan, Loïc Brunet, Christian Slomianny, and Rabah Boukherroub. "Cumulative Effect of Zinc Oxide and Titanium Oxide Nanoparticles on Growth and Chlorophyll a Content of *Picochlorum* sp." *Environmental Science and Pollution Research* 23, no. 3 (2016): 2821-30.

Honey, Martha, and David Krantz. "Global Trends in Coastal Tourism." *Washington DC: Center on Ecotourism and Sustainable Development* (2007).

Horio, T, and T Higuchi. "Photocontact Dermatitis from P-Aminobenzoic Acid." *Dermatology* 156, no. 2 (1978): 124-28.

Johnston, Emma L, and David A Roberts. "Contaminants Reduce the Richness and Evenness of Marine Communities: A Review and Meta-Analysis." *Environmental Pollution* 157, no. 6 (2009): 1745-52.

Jovanović, Boris, and Héctor M Guzmán. "Effects of Titanium Dioxide (Tio2) Nanoparticles on Caribbean Reef-Building Coral (*Montastraea faveolata*)." *Environmental Toxicology and Chemistry* 33, no. 6 (2014): 1346-53.

Juganson, Katre, Angela Ivask, Irina Blinova, Monika Mortimer, and Anne Kahru. "Nano-eTox: New and in-Depth Database Concerning Ecotoxicity of Nanomaterials." *Beilstein Journal of Nanotechnology* 6 (2015): 1788.

Karlsson, Isabella, Lisa Hillerström, Anna-Lena Stenfeldt, Jerker Mårtensson, and Anna Börje. "Photodegradation of Dibenzoylmethanes: Potential Cause of Photocontact Allergy to Sunscreens." *Chemical Research in Toxicology* 22, no. 11 (2009): 1881-92.

Keller, Arturo A, Hongtao Wang, Dongxu Zhou, Hunter S Lenihan, Gary Cherr, Bradley J Cardinale, Robert Miller, and Zhaoxia Ji. "Stability and Aggregation of Metal Oxide Nanoparticles in Natural Aqueous Matrices." *Environmental Science & Technology* 44, no. 6 (2010): 1962-67.

Kiser, MA, P Westerhoff, T Benn, Y Wang, J Perez-Rivera, and K Hristovski. "Titanium Nanomaterial Removal and Release from Wastewater Treatment Plants." *Environmental Science & Technology* 43, no. 17 (2009): 6757-63.

Kusk, Kresten Ole, Manola Avdolli, and Leah Wollenberger. "Effect of 2, 4-Dihydroxybenzophenone (Bp1) on Early Life-Stage Development of the Marine Copepod *Acartia tonsa* at Different Temperatures and Salinities." *Environmental Toxicology and Chemistry* 30, no. 4 (2011): 959-66.

Lanzante, John R. “A Cautionary Note on the Use of Error Bars.” *Journal of Climate* 18, no. 17 (2005): 3699-3703.

Lesser, Michael P. “Oxidative Stress in Marine Environments: Biochemistry and Physiological Ecology.” *Annu. Rev. Physiol.* 68 (2006): 253-78.

Li, Weihong, Yongmin Ma, Changsheng Guo, Wei Hu, Keming Liu, Yuqiu Wang, and Tan Zhu. “Occurrence and Behavior of Four of the Most Used Sunscreen UV Filters in a Wastewater Reclamation Plant.” *Water Research* 41, no. 15 (2007): 3506-12.

Libralato, Giovanni, Diego Minetto, Sara Totaro, Ivan Mičetić, Andrea Pigozzo, Enrico Sabbioni, Antonio Marcomini, and Annamaria Volpi Ghirardini. “Embryotoxicity of TiO2 Nanoparticles to *Mytilus galloprovincialis* (Lmk).” *Marine Environmental Research* 92 (2013): 71-78.

Lowe, N. “An Overview of Ultraviolet Radiation, Sunscreens, and Photo-Induced Dermatoses.” *Dermatologic Clinics* 24, no. 1 (2006): 9-17.

MacManus-Spencer, Laura A, Monica L Tse, Jacob L Klein, and Alison E Kracunas. “Aqueous Photolysis of the Organic Ultraviolet Filter Chemical Octyl Methoxycinnamate.” *Environmental Science & Technology* 45, no. 9 (2011): 3931-37.

Maipas, Sotirios, and Polyxeni Nicolopoulou-Stamati. “Sun Lotion Chemicals as Endocrine Disruptors.” *Hormones* 14, no. 1 (2015): 32-46.

Manaia, Eloísa Berbel, Renata Cristina Kiatkoski Kaminski, Marcos Antonio Corrêa, and Leila Aparecida Chiavacci. “Inorganic UV Filters.” *Brazilian Journal of Pharmaceutical Sciences* 49, no. 2 (2013): 201-09.

Manzo, Sonia, Maria Lucia Miglietta, Gabriella Rametta, Silvia Buono, and Girolamo Di Francia. “Toxic Effects of ZnO Nanoparticles Towards Marine Algae *Dunaliella tertiolecta*.” *Science of The Total Environment* 445 (2013): 371-76.

Margot, Jonas, Luca Rossi, David A Barry, and Christof Holliger. “A Review of the Fate of Micropollutants in Wastewater Treatment Plants.” *Wiley Interdisciplinary Reviews: Water* 2, no. 5 (2015): 457-87.

McCoshum, Shaun M, Alicia M Schlarb, and Kristen A Baum. “Direct and Indirect Effects of Sunscreen Exposure for Reef Biota.” *Hydrobiologia* (2016): 1-8.

Medina, Matías H, Juan A Correa, and Carlos Barata. “Micro-Evolution Due to Pollution: Possible Consequences for Ecosystem Responses to Toxic Stress.” *Chemosphere* 67, no. 11 (2007): 2105-14.

Miao, Ai-Jun, Xue-Yin Zhang, Zhiping Luo, Chi-Shuo Chen, Wei-Chun Chin, Peter H Santschi, and Antonietta Quigg. "Zinc Oxide–Engineered Nanoparticles: Dissolution and Toxicity to Marine Phytoplankton." *Environmental Toxicology and Chemistry* 29, no. 12 (2010): 2814-22.

Miglietta, M. L., G. Rametta, G. Di Francia, S. Manzo, A. Rocco, R. Carotenuo, F. De Luca Picione, and S. Buono. "Characterization of Nanoparticles in Seawater for Toxicity Assessment Towards Aquatic Organisms" *Sensors and Microsystems*, (2011): 425-429.

Miller, Margaret W, Kathryn E Lohr, Caitlin M Cameron, Dana E Williams, and Esther C Peters. "Disease Dynamics and Potential Mitigation among Restored and Wild Staghorn Coral, *Acropora cervicornis*." *PeerJ* 2 (2014): e541.

Miller, Robert J, Samuel Bennett, Arturo A Keller, Scott Pease, and Hunter S Lenihan. "TiO2 Nanoparticles Are Phototoxic to Marine Phytoplankton." *PloS One* 7, no. 1 (2012): e30321.

Miller, Robert J, Hunter S Lenihan, Erik B Muller, Nancy Tseng, Shannon K Hanna, and Arturo A Keller. "Impacts of Metal Oxide Nanoparticles on Marine Phytoplankton." *Environmental Science & Technology* 44, no. 19 (2010): 7329-34.

Morgan, Michael B, Dale L Vogelien, and Terry W Snell. "Assessing Coral Stress Responses Using Molecular Biomarkers of Gene Transcription." *Environmental Toxicology and Chemistry* 20, no. 3 (2001): 537-43.

Nakajima, R, and Y Tanaka. "The Role of Coral Mucus in the Material Cycle in Reef Ecosystems: Biogeochemical and Ecological Perspectives." *J. Jpn. Coral Reef Soc* 16 (2014): 3-27.

Nash, J Frank, and Paul R Tanner. "Relevance of UV Filter/Sunscreen Product Photostability to Human Safety." *Photodermatology, Photoimmunology & Photomedicine* 30, no. 2-3 (2014): 88-95.

Newman, Michael C. *Fundamentals of Ecotoxicology*. CRC press, 2009.

Osterwalder, Uli, Myriam Sohn, and Bernd Herzog. "Global State of Sunscreens." *Photodermatology, Photoimmunology & Photomedicine* 30, no. 2-3 (2014): 62-80.

Paredes, E, S Perez, R Rodil, JB Quintana, and R Beiras. "Ecotoxicological Evaluation of Four UV Filters Using Marine Organisms from Different Trophic Levels *Isochrysis galbana*, *Mytilus galloprovincialis, Paracentrotus lividus,* and *Siriella armata*." *Chemosphere* 104 (2014): 44-50.

Peng, Xiaohui, Shelagh Palma, Nicholas S Fisher, and Stanislaus S Wong. "Effect of Morphology of ZnO Nanostructures on Their Toxicity to Marine Algae." *Aquatic Toxicology* 102, no. 3 (2011): 186-96.

Peters, EC, KL Price, and DJ Borsay Horowitz. "Histological Preparation of Invertebrates for Evaluating Contaminant Effects." *Techniques in Aquatic Toxicology* 2 (2005): 653-86.

Peters, Esther C, Nancy J Gassman, Julie C Firman, Robert H Richmond, and Elizabeth A Power. "Ecotoxicology of Tropical Marine Ecosystems." *Environmental Toxicology and Chemistry* 16, no. 1 (1997): 12-40.

Petersen, Karina, Harald Hasle Heiaas, and Knut Erik Tollefsen. "Combined Effects of Pharmaceuticals, Personal Care Products, Biocides and Organic Contaminants on the Growth of *Skeletonema pseudocostatum*." *Aquatic Toxicology* 150 (2014): 45-54.

Plagellat, Cécile, Thomas Kupper, Reinhard Furrer, Luiz Felippe De Alencastro, Dominique Grandjean, and Joseph Tarradellas. "Concentrations and Specific Loads of UV Filters in Sewage Sludge Originating from a Monitoring Network in Switzerland." *Chemosphere* 62, no. 6 (2006): 915-25.

Poiger, Thomas, Hans-Rudolf Buser, Marianne E Balmer, Per-Anders Bergqvist, and Markus D Müller. "Occurrence of UV Filter Compounds from Sunscreens in Surface Waters: Regional Mass Balance in Two Swiss Lakes." *Chemosphere* 55, no. 7 (2004): 951-63.

Printz, Carrie. "Sunscreen Slow Down: The US FDA's Sluggish Approval of New Sunscreens Frustrates Physicians, Manufacturers, and Consumers." *Cancer* 121, no. 17 (2015): 2861-62.

Ramos, Sara, Vera Homem, Arminda Alves, and Lúcia Santos. "A Review of Organic UV-Filters in Wastewater Treatment Plants." *Environment International* 86 (2016): 24-44.

Rebut, D. "The Sunscreen Industry in Europe: Past, Present, and Future." In *Sunscreens: Development, Evaluation and Regulatory Aspects*, 161-71: Marcel Dekker, Inc, New York, 1990.

Rigel, Darrell S, Robert A Weiss, Henry W Lim, and Jeffrey S Dover. *Photoaging*. CRC Press, 2004.

Rinkevich, Baruch. "Conservation of Coral Reefs through Active Restoration Measures: Recent Approaches and Last Decade Progress." *Environmental Science & Technology* 39, no. 12 (2005): 4333-42.

Ritter, Keith Solomon, Paul Sibley, Ken Hall, Patricia Keen, Gevan Mattu, Beth Linton, Len. "Sources, Pathways, and Relative Risks of Contaminants in Surface Water and Groundwater: A Perspective Prepared for the Walkerton Inquiry." *Journal of Toxicology and Environmental Health Part A* 65, no. 1 (2002): 1-142.

Salvador, A, and A Chisvert. "Sunscreen Analysis: A Critical Survey on UV Filters' Determination." *Analytica Chimica Acta* 537, no. 1 (2005): 1-14.

Sánchez-Quiles, David, and Antonio Tovar-Sánchez. "Sunscreens as a Source of Hydrogen Peroxide Production in Coastal Waters." *Environmental Science & Technology* 48, no. 16 (2014): 9037-42.

Sánchez-Quiles, David, and Antonio Tovar-Sánchez. "Are Sunscreens a New Environmental Risk Associated with Coastal Tourism?". *Environment International* 83 (2015): 158-70.

Schiavo, S, M Oliviero, M Miglietta, G Rametta, and S Manzo. "Genotoxic and Cytotoxic Effects of ZnO Nanoparticles for *Dunaliella tertiolecta* and Comparison with SiO2 and TiO2 Effects at Population Growth Inhibition Levels." *Science of the Total Environment* 550 (2016): 619-27.

Schrager, TF. "What is Toxicology". *online: ToxicologySource Homepage<www.toxicologysource.com/whatistoxicology.html.* (2006).

Schrurs, Françoise, and Dominique Lison. "Focusing the Research Efforts." *Nature Nanotechnology* 7, no. 9 (2012): 546-48.

Sharp, Koty et al. "Effects of Benzophenone-3 and Bulk Titanium Dioxide on *Porites astreoides* Planulae". *Unpublished manuscript* (2015).

Sharfstein, Joshua M. "A Spotlight on Sunscreen Regulation." *New England Journal of Medicine* 373, no. 2 (2015): 101-03.

Sikes, Ruth G. "The History of Suntanning: A Love/Hate Affair." *Journal of Aesthetic Sciences* 1, no. 2 (1998): 1-7.

Skelly, Claire W, Jordyn LeBlonde, and Salwa El Khattabi-Salazar. "The Effects of Sunscreen on Coral Bleaching of the Genus Pocillopora Located in Baja California Sur." *Independent Research Projects* (2012): 76.

Skocaj, Matej, Metka Filipic, Jana Petkovic, and Sasa Novak. "Titanium Dioxide in Our Everyday Life; Is It Safe?". *Radiology and Oncology* 45, no. 4 (2011): 227-47.

Sobek, Anna, S Bejgarn, C Rudén, Linda Molander, and Magnus Breitholtz. "In the Shadow of the Cosmetic Directive—Inconsistencies in EU Environmental Hazard

Classification Requirements for UV-Filters." *Science of the Total Environment* 461 (2013): 706-11.

Spisni, E, S Seo, SH Joo, and C Su. "Release and Toxicity Comparison between Industrial-and Sunscreen-Derived Nano-ZnO Particles." *International Journal of Environmental Science and Technology* 13, no. 10 (2016): 2485-94.

Stengel, D, Sarah O'Reilly, and John O'Halloran. "Contaminants and Pollutants." In *The Ecology of Transportation: Managing Mobility for the Environment*, 361-89: Springer, 2006.

Sulzberger, Marion B, Abram Kanof, Rudolf L Baer, and Clare Lowenberg. "Sensitization by Topical Application of Sulfonamides." *Journal of Allergy* 18, no. 2 (1947): 92-103.

Suter, Glenn W. "A Critique of Ecosystem Health Concepts and Indexes." *Environmental Toxicology and Chemistry* 12, no. 9 (1993): 1533-1539.

Suman, TY, SR Radhika Rajasree, and R Kirubagaran. "Evaluation of Zinc Oxide Nanoparticles Toxicity on Marine Algae *Chlorella vulgaris* through Flow Cytometric, Cytotoxicity and Oxidative Stress Analysis." *Ecotoxicology and Environmental Safety* 113 (2015): 23-30.

Tovar-Sánchez, Antonio, David Sánchez-Quiles, Gotzon Basterretxea, Juan L Benedé, Alberto Chisvert, Amparo Salvador, Ignacio Moreno-Garrido, and Julián Blasco. "Sunscreen Products as Emerging Pollutants to Coastal Waters." *PLoS One* 8, no. 6 (2013): e65451.

Tsui, Mirabelle MP, HW Leung, Tak-Cheung Wai, Nobuyoshi Yamashita, Sachi Taniyasu, Wenhua Liu, Paul KS Lam, and Margaret B Murphy. "Occurrence, Distribution and Ecological Risk Assessment of Multiple Classes of UV Filters in Surface Waters from Different Countries." *Water Research* 67 (2014): 55-65.

"Understanding Toxic Substances". CDPH, California Department of Public Health. 1986. www.cdph.ca.gov/Programs/CCDPHP/DEODC/OHB/HESIS/CDPH%20Document%20Library/introtoxsubstances.pdf#search=introduction%20to%20toxic%20substances (accessed March 17, 2018).

U.S. Fish and Wildlife Service Environmental Conservation Online System. https://ecos.fws.gov/ecp0/profile/speciesProfile?spcode=P000 (accessed March 17, 2018).

Urbach, Frederick. "The Historical Aspects of Sunscreens." *Journal of Photochemistry and Photobiology B: Biology* 64, no. 2 (2001): 99-104.

Wang, Steven Q, Jacqueline M Goulart, and Henry W Lim. "Lack of UV-a Protection in Daily Moisturizing Creams." *Archives of Dermatology* 147, no. 5 (2011): 618-20.

Wang, Steven Q, and Judy Y Hu. "Challenges in Making Effective Sunscreen." *The Melanoma Letter* 30 (2012): 4-6.

Wang, Yixiang, Xiaoshan Zhu, Yongmin Lao, Xiaohui Lv, Yi Tao, Boming Huang, Jiangxin Wang, Jin Zhou, and Zhonghua Cai. "TiO2 Nanoparticles in the Marine Environment: Physical Effects Responsible for the Toxicity on Algae *Phaeodactylum tricornutum*." *Science of The Total Environment* 565 (2016): 818-26.

Wong, Stella WY, Priscilla TY Leung, AB Djurišić, and Kenneth MY Leung. "Toxicities of Nano Zinc Oxide to Five Marine Organisms: Influences of Aggregate Size and Ion Solubility." *Analytical and Bioanalytical Chemistry* 396, no. 2 (2010): 609-18.

Xia, Bin, Bijuan Chen, Xuemei Sun, Keming Qu, Feifei Ma, and Meirong Du. "Interaction of TiO2 Nanoparticles with the Marine Microalga Nitzschia Closterium: Growth Inhibition, Oxidative Stress and Internalization." *Science of the Total Environment* 508 (2015): 525-33.

Xin, Loke H, Kee AA Adzis, Julian Hyde, and Zaidi C Cob. "Growth Performance of *Acropora formosa* in Natural Reefs and Coral Nurseries for Reef Restoration." *Aquaculture, Aquarium, Conservation & Legislation-International Journal of the Bioflux Society (AACL Bioflux)* 9, no. 5 (2016).

Young, CN, SA Schopmeyer, and Diego Lirman. "A Review of Reef Restoration and Coral Propagation Using the Threatened Genus Acropora in the Caribbean and Western Atlantic." *Bulletin of Marine Science* 88, no. 4 (2012): 1075-98.

Yung, Mana MN, Stella WY Wong, Kevin WH Kwok, FZ Liu, YH Leung, WT Chan, XY Li, AB Djurišić, and Kenneth MY Leung. "Salinity-Dependent Toxicities of Zinc Oxide Nanoparticles to the Marine Diatom *Thalassiosira pseudonana*."*Aquatic Toxicology* 165 (2015): 31-40.

Zhang, Cai, Jiangtao Wang, Liju Tan, and Xiaohua Chen. "Toxic Effects of Nano-ZnO on Marine Microalgae *Skeletonema costatum*: Attention to the Accumulation of Intracellular Zn." *Aquatic Toxicology* 178 (2016): 158-64.

Zhang, Zifeng, Nanqi Ren, Yi-Fan Li, Tatsuya Kunisue, Dawen Gao, and Kurunthachalam Kannan. "Determination of Benzotriazole and Benzophenone UV Filters in Sediment and Sewage Sludge." *Environmental Science & Technology* 45, no. 9 (2011): 3909-16.

Zhu, Xiaoshan, Jin Zhou, and Zhonghua Cai. “The Toxicity and Oxidative Stress of TiO2 Nanoparticles in Marine Abalone (*Haliotis diversicolor supertexta*).” *Marine Pollution Bulletin* 63, no. 5 (2011): 334-38.