Impacts of Cosmetic Ingredients on Larval Barnacles:
A Study & Discussion of How Cosmetic Ingredients Affect Marine Life

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ABSTRACT

In recent years, scientists have discovered toxicity of active ingredients in sunscreens to marine life such as coral reefs. While the research findings have brought about significant policy changes in places like Hawaii and Palau, little attention has been given to the hundreds of other ingredients that are commonly found in such cosmetics. Through 24-hour exposure studies with barnacle larvae, this study documents toxicity and settlement inhibition of sunscreens and 5 common "inactive" ingredients. Results indicate that "inactive PDMS additives" are toxic to barnacle larvae inhibit settlement. Sunscreens are even more potent, therefore suggesting the combination of ingredients that makeup sunscreens pose a greater risk to marine life than individual components. The Master's Project products are an analysis and discussion of the research conducted, as well as a short video translating the research for science communication purposes.

INTRODUCTION

Tourism & Physical Threats

As disposable income has increased with strengthening economies worldwide, so has leisure time and tourism (Jobbins, 2006) to over 1.326 billion travelers (World Tourism Organization, 2018). In the realm of water-based tourism, even remote coastal destinations are facing an inundation of tourists, resulting in more development and beachgoers every year (Jobbins, 2006).

As tourism increased, researchers began investigating the anthropogenic effects associated with recreation. For example, the greatest environmental threat from the increase in dive tourists is their physical contact with the coral reefs. Tourists stir up sediment and break coral fragments with their fins. Coral damage is increasing so much that there is concern over how sustainable dive tourism can be (Barker & Roberts, 2004). This is just one specific example of the physical impacts that tourists bring to the marine environment. Development of resorts to accommodate tourists leads to increased levels of chemical runoff from the resorts and from tourists themselves (Jobbins, 2006).

Tourism & Chemical Pollution: Sunscreens

Although nutrient pollution is a major issue, another significant focus of chemical runoff concerns in coastal tourism is from sunscreens and the UV absorbing chemicals that they contain. Between 6,000 and 14,000 tons of sunscreens enter the marine environment each year (Downs, et al., 2016). The active ingredients in sunscreens have been listed as a new threat to marine life (Downs, et al., 2016). Recent research has discovered a primary active ingredient in sunscreens, oxybenzone, is toxic to coral (Danovaro, et al., 2008; Downs, et al., 2016). Delivery of UV absorbing chemicals from beachgoing and diving tourists' sunscreens are toxic to coral larvae, planulae, and increase coral bleaching rates (Danovaro, et al., 2008; Downs, et al., 2016). Sunscreen chemicals can enter the marine environment through leeching from skin with substantial delivery of absorbed molecules in sunscreen users' urine (National Center for Biotechnology Information, n.d.).

These findings, as well as similar research on other UV absorbing chemicals commonly in sunscreens, has raised awareness to the greater public, especially those reliant on coral reef health for tourism and fisheries. Hawaii was the first state to ban the sale of sunscreens containing oxybenzone and octinoxate (Environmental Working Group, 2018). Next, Palau banned both oxybenzone and octinoxate, as well as eight other ingredients commonly found in sunscreens (McGrath, 2018). These bans are profound examples of how scientific research can inform policy.

Other Ingredients in Sunscreens

While there has been a large focus on the impact of active ingredients in cosmetics on the marine environment, there is little study of the "inactive ingredients". Why are they called inactive? Such ingredients are used in sunscreens, makeup, lotions and shampoos for purposes such as increasing the product's shelf life, keeping the product from separating, adding scent, and spreading evenly across skin or hair (Australian Academy of Science). The most common ingredient added to lotions to aid in spreading and hydration is polydimethylsiloxane (PDMS) (Owen, 2001).

PDMS

Polydimethylsiloxane is primarily used to create a hydration barrier on the skin or hair (Fowler, 2000; Silicones Health And Safety Council of North America, Centre Europeen Des Silicones, Silicone Industry Association of Japan). PDMS is used for a variety of products outside of personal care products, such as food additives, antifoaming agents, and medicinal products (Stevens C. , Powell, Makela, & Karman, 2001).

Also known as dimethicone or a silicone, polydimethylsiloxane is mostly found in the marine environment due to consumer product runoff and wastewater (Stevens C. , Powell, Makela, & Karman, 2001). Based on toxicology research, dimethicone does not appear to have concerning impacts to marine life (Silicones Health And Safety Council of North America, Centre Europeen Des Silicones, Silicone Industry Association of Japan). On the other hand, the substrates, contaminants and breakdown products associated with PDMS have received little attention.

PDMS is polymerized from silicone compounds such as cyclotrisiloxane (D3), cyclotetrasiloxane (D4), and cyclopentasiloxane (D5), using high heat and Palladium and Activated Carbon and Cerium Oxide as catalysts (Kulyk, et al., 2016; Elkins & Long, 2004; The Danish Environmental Protection Agency, 2014). Yields from these reactions are 70-80% PDMS, with the initial ingredients and catalysts in the remaining 20-30%. This complex mixture is used in PDMS-based cosmetics (Elkins & Long, 2004).

When PDMS is exposed to heat, such as when a bottle of sunscreen is left in a car on a hot, sunny day, some proportion breaks down to a volatile substance dimethylsilanone (Kulyk, et al., 2016). While PDMS remains well-studied, D3, D4, D5, the catalysts, and dimethylsilanone are not.

Goals of Project

This Master's Project was designed to probe toxicity and settlement inhibition of the substrates and breakdown products associated with PDMS on barnacle larvae and to translate research for community education and outreach. Compounds and mixtures were tested using a well-studied marine animal model system, barnacles, *Amphibalanus* (=Balanus) *amphitrite* (Rittschof & Clare, 1992).

The following substrates were selected for this project: D4, D5, Palladium on Activated Carbon, Cerium Oxide, and Dimethylsilanone. In additional experiments, I tested the complex commercial products: Banana Boat Sport Performance SPF 50+ (Banana Boat, n.d.), Sun Bum SPF 70 (Sun Bum, n.d.), Neutrogena Ultra Sheer Dry-Touch SPF 45 (Neutrogena, n.d.), and Neutrogena Beach Defense SPF 30 (Neutrogena, n.d.).

RESEARCH METHODOLOGY

Research Questions & Objectives

The following were the main research questions:

- How toxic are complex mixtures known as sunscreens?
- Do silicone components impact barnacle larvae?
- Are there implications for cosmetics?
- How can I communicate this research to the general public?

Barnacle Acquisition

Barnacle larvae, *Amphibalanus (=Balanus) amphitrite amphitrite* were chosen as the subject for this research for multiple reasons. From a marine toxicology standpoint, barnacles have been studied for over 35 years (Standing, Hooper, & Costlow, 1984; Rittschof, Branscomb, & Costlow, 1984). Among the various species of barnacles, they reproduce nearly once per week, easily allowing for multiple experiments (Rittschof & Clare, 1992). Finally, from an ecological standpoint, barnacles are prey for the greater marine food web, filter feeding animals, whelks, sea stars, and small fish prey on them (Australasia, n.d.).

My methods are based on previous barnacle larvae toxicity and settlement research (Rittschof & Clare, 1992; Rittschof, Branscomb, & Costlow, 1984; Sasikumar, Clare, Gerhart, Stover, & Rittschof, 1995). Locally collected adult barnacles maintained in a laboratory were used as broodstock and cultured as described in Rittschof et al., 1992.

Toxicity Assays

For toxicity bioassays, broodstock were incubated overnight at 28°C in air and induced to release brooded nauplii with addition of 28°C water and a small amount of cultured algae. Stage II nauplii, were collected into a fresh container of 800 mL of aged filtered sea water between 9 AM and 12 PM. Collection was in a dark room using a point source of light to attract nauplii to the walls of the broodstock container. Larvae were held at 25°C without aeration 2 to 4 hours until use in toxicity assays. Nauplii were concentrated with a point source of light and pipetted into test tubes containing dilution series of the test compounds.

For testing, test tubes were prepared in triplicate, in the following concentrations: 1.0 mg/mL, 0.1 mg/mL, and 0.01 mg/mL, each in 3 mL of filtered age sea water. Filtered aged seawater was used for dilutions. Approximately 20 nauplii were added to each test tube. A control of three tubes of filtered aged sea water without additions was included in each trial. Assay tubes with larvae incubated for 24 hours at 28°C with a 12:12 L:D cycle.

After incubation the numbers of swimming (live) and non-swimming (dead) nauplii in each sample were counted. This was conducted under a microscope using a Bogorov tray. The numbers of dead larvae and alive larvae were used to calculate percentage dead and determine the LC_{50}, the concentration of a test substance that killed 50% of the larvae.

Settlement Assays

Settlement stage barnacle larvae were tested for inhibition of settlement with test compounds. Nauplii were cultured to settlement stage, aged for 3 days at 6°C and then used in settlement assays (Rittschof & Clare, 1992; Rittschof, Branscomb, & Costlow, 1984). Settlement assays used the same design as toxicity assays with nauplii. Glass tubes were used instead of plastic dishes, as many ingredients tested would bind to the plastic dishes used in standard settlement assays. A control group of aged seawater was used in each trial for comparison. Approximately 10-20 cyprids were collected for each test tube and incubated for 24 hours at 28°C. Using a microscope, results were quantified to determine the number of attached (or settled) cyprids versus unattached (or unsettled) cyprids. For these trials, the amount of unsettled cyprids were considered to determine the concentration that results in 50% inhibited settlement (Rittschof & Clare, 1992).

RESULTS

Sunscreen Lotion Toxicity Assays

In order to understand the effects of the additive and breakdown products associated with PDMS compared to the overall effects of the sunscreen lotions they make up, toxicity assays and settlement assays were conducted using four common sunscreens: Banana Boat Sport Performance SPF 50+ (Banana Boat, n.d.), Sun Bum SPF 70 (Sun Bum, n.d.), Neutrogena Ultra Sheer Dry-Touch SPF 45 (Neutrogena, n.d.), and Neutrogena Beach Defense SPF 30 (Neutrogena, n.d.).

All tested sunscreens increased mortality rates compared to the control (*Figure 1*). In all concentrations, mortality rate did not reach below 80% for Banana Boat Sport Performance, or below 70% for Neutrogena Ultra Sheer Dry-Touch or Neutrogena Beach Defense. Sun Bum was least toxic out of the four tested sunscreen lotions, with a 48% dead nauplii at 0.01 mg/mL. Banana Boat and Neutrogena Ultra Sheer Dry-Touch led to 100% mortality in one or more concentrations tested.

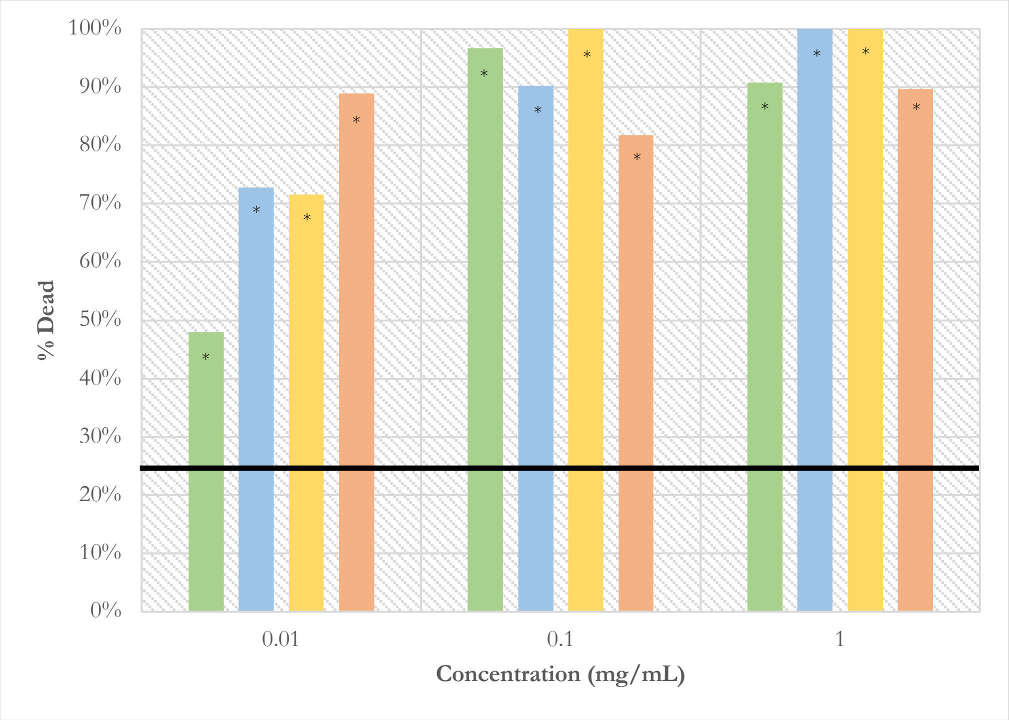

Figure 1. Death of nauplii after 24-hour exposure to different concentrations of common sunscreen lotions. Green: Sun Bum SPF 70; Blue: Neutrogena Beach Defense SPF 30; Yellow: Neutrogena Ultra Sheer Dry-Touch SPF 45; Orange: Banana Boat Sport Performance SPF 50+.
* significance at $p<0.01$, ** significant at $p<0.05$.

Sunscreen Lotion Settlement Assays

Every sunscreen tested inhibited settlement of cyprids compared to the control (*Figure 2*). In particular, as the concentration of Banana Boat Sport Performance SPF 50+ increased, settlement increased as well, though always less than the control. The three other sunscreens tested appeared to have an inverse relationship, decreasing settlement rates as concentrations increased.

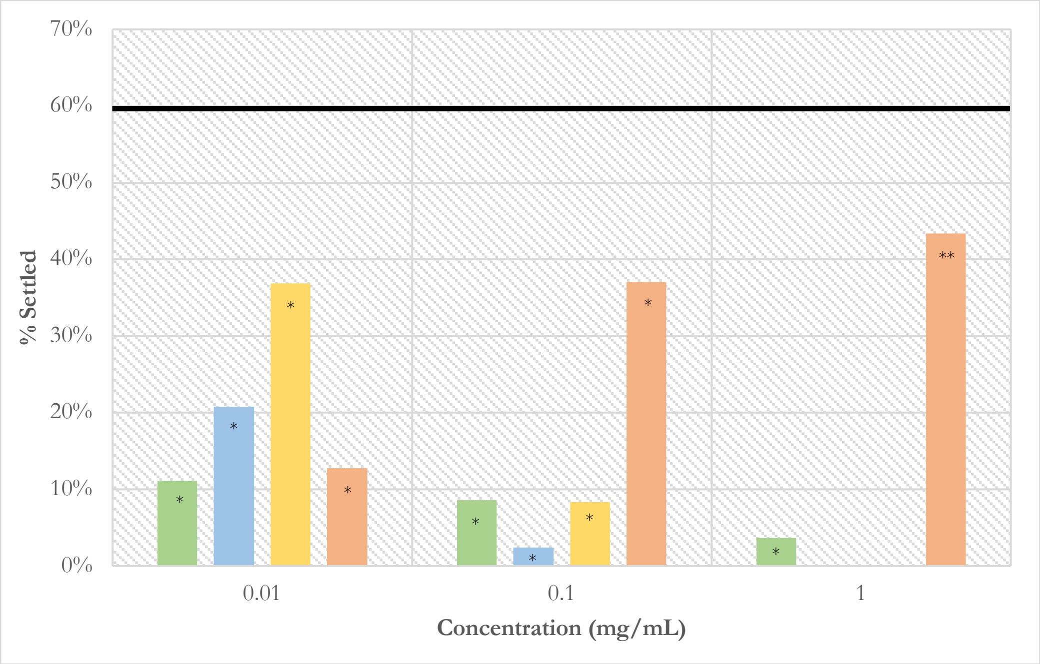

Figure 2. Settlement of cyprids after 24-hour exposure to different concentrations of common sunscreen lotions. Green: Sun Bum SPF 70; Blue: Neutrogena Beach Defense SPF 30; Yellow: Neutrogena Ultra Sheer Dry-Touch SPF 45; Orange: Banana Boat Sport Performance SPF 50+.
* significance at $p<0.01$, ** significant at $p<0.05$.

Additive & Breakdown Product Toxicity Assays

Mortality of nauplii exposed to components associated with PDMS is shown in *Figure 3*. The breakdown product, dimethylsilanone, promoted survival compared to control at low doses. All but D5 were slightly more toxic than controls at all test dilutions. Only D4 caused high mortality at the highest concentration tested. Aside from dimethylsilanone, most ingredients led to higher mortality than the control. Cyclotetrasiloxane, or D4, was the most significant, resulting in 100% mortality at 1.0 mg/mL concentration.

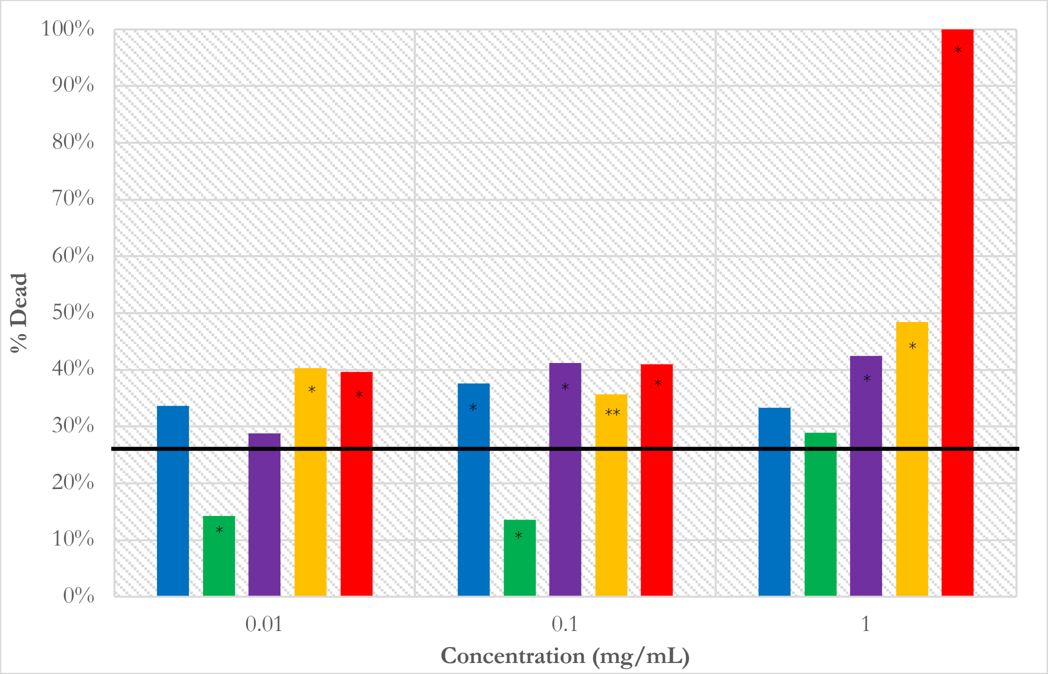

Figure 3. Death of nauplii after 24-hour exposure to different concentrations of potential additives and breakdown products of PDMS. Black Bar: Aged Seawater (Control); Blue: Palladium on Activated Carbon; Green: Dimethylsilanone; Purple: Cerium Oxide; Yellow: D5; Red: D4.
* significance at $p<0.01$, ** significant at $p<0.05$.

Additive & Breakdown Product Settlement Assays

After 24-hours of exposure to the substrates associated with PDMS, settlement rates of cyprids were compared to a control (*Figure 4*). Four of the five substances (D5, dimethylsilanone, cerium oxide, and palladium on activated carbon) inhibited settlement at all concentrations tested, though especially at 1.0 mg/mL.

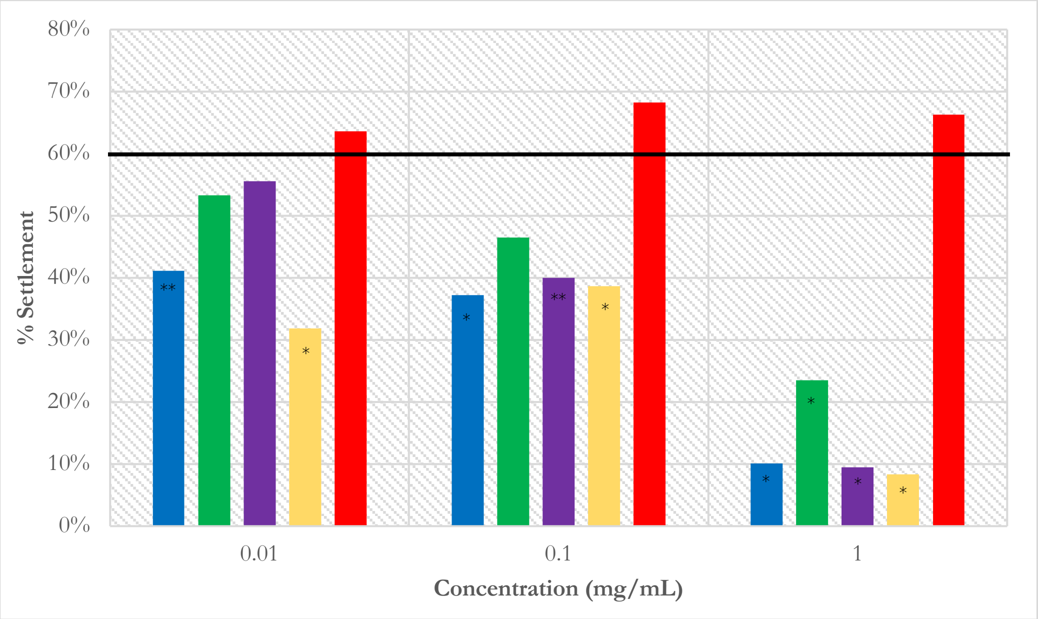

Figure 4. Settlement of cyprids after 24-hour exposure to different concentrations of potential additives and breakdown products of PDMS. Blue: Palladium on Activated Carbon; Green: Dimethylsilanone; Purple: Cerium Oxide; Yellow: D5; Red: D4.
* significance at p<0.01, ** significant at p<0.05.

Unlike the other four ingredients tested, D4, or cyclotetrasiloxane, induced settlement of barnacle cyprids compared to the control rate. Based on the results of both the toxicity and settlement assays, it appears D4 reduces survival rate of nauplii, yet increases survival rate of cyprids (*Figure 5*).

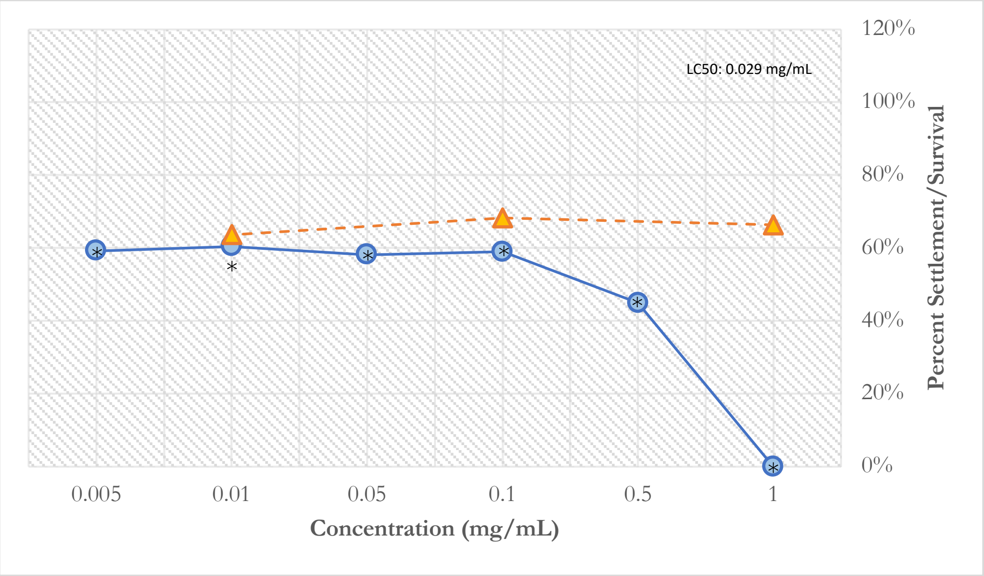

Figure 5. Survival of Nauplius and Settlement of Cyprids after 24-hour exposure to different concentrations of D4. Blue: Survival Rate, Orange: Settlement Rate.
* significance at p<0.01, ** significant at p<0.05.

As noted in Figure 5, the LC_{50} for D4 calculated through Probit analysis is approximately 0.03 mg/mL. Compared to the LC_{50} of three of the four sunscreens tested, however, Neutrogena Beach Defense is 0.005 mg/mL, Sun Bum is 0.003 mg/mL, and Neutrogena Ultra Sheer Dry-Touch is 0.002 mg/mL. Thus, these complex mixtures that are sunscreens are around 6 to 18-fold more toxic than pure D4.

DISCUSSION

Banana Boat Performance was the only sunscreen that had a positive relationship compared to the other sunscreen counterparts, increasing settlement rates as concentrations increased. However, the settlement rates were still below that of the control. This is possibly due to the chemical properties of Banana Boat Performance that leads to the product being highly water and sweat resistant. Since the sunscreen is less able to dilute into the water, thus minimizing its chances of exposure with barnacle larvae. However, to confirm this hypothesis, further research including chemical analysis on more sunscreens that are water resistant and not water resistant would be needed.

As noted in the additive and breakdown product assays, cyclotetrasiloxane, D4, led to the most significant mortality rate of barnacle nauplii yet increased settlement rates of barnacle cyprids. It is unclear what led to these opposite reactions. One possibility is settlement which takes only 6 to 8 hours, occurred before the 24-hour toxic effects manifested. Thus, for sunscreens and other cosmetics that contain dimethicone made using D4, the runoff of such cosmetics in the marine environment can reduce the abilities for barnacles to successfully recruit.

When comparing the results of the additive and breakdown product assays to the sunscreen lotion assays, it is evident that the individual ingredients tested are not the only ones impacting survival and settlement rates of barnacle larvae. Perhaps other inactive ingredients lead to increased potency. Likely candidates are emulsifiers and preservatives, both of which are known toxicants. Another option is the cumulative effects of mixtures of toxicants. Further research would be required to pinpoint additional ingredients, as there are numerous in each cosmetic product.

Considering the ingredients and products tested here raises more questions over the impacts of heavy water-based tourism and cosmetic use around these destinations. While all sunscreens contain PDMS or related products, it is evident that PDMS-related ingredients negatively impact barnacle larvae.

Implications for Sunscreens

The overall combination of ingredients that makeup sunscreens is significantly more concerning than the individual PDMS ingredients tested. Sunscreen manufacturers should be forced to consider the environmental impact of not just their individual ingredients, but the overall products prior to marketing them. Consumers should request environmental impact assessments from their preferred cosmetic companies in addition to shopping for sunscreens that are approved by Environmental Working Group.

Implications of PDMS Use

PDMS and ingredients are the least of sunscreen and lotion concerns. While this research may lead a consumer to consider using products that do not contain the harmful additive ingredients deemed toxic, it is extremely difficult to snuff that out. Since there are thousands of patents for PDMS, each with different ingredient makeups and production methods, it is unclear how many sunscreens and other cosmetics on the market in fact contain ingredients like D4.

Additional Experiments Required Prior to Publication

Prior to submitting this research paper for publication, further experiments will be conducted. The first experiments will use a new set of dilution series for the four sunscreen products, at 0.001 mg/mL and 0.0001 mg/mL. This will lead to a better understanding of the range of toxicity and settlement impacts from each product and will enable determination of effective concentrations for 50% toxicity and 50% settlement inhibition. Secondly, larval barnacle toxicity and settlement tests will be conducted with four sunscreens that are marketed as "coral reef-friendly" and contain mineral-based UV filters like titanium dioxide and zinc oxide (Sun Bum Mineral Sunscreen, Alba Sensitive Mineral Sunscreen, Babo Botanicals Clear Zinc Sunscreen, Bare Republic Mineral SPF 50 Sport Sunscreen Lotion). This will enable comparison of effective concentrations of all 8 complex mixtures. Doing so will allow for a comparison of toxicity and settlement rates between the sunscreens already seen as toxic to corals versus those that are marketed as safer alternatives. As barnacles and barnacle larvae and integral parts of most marine ecosystems, these are relevant and revealing tests.

Legal Implications

While this Master's Project research is only one example of how cosmetic ingredients affect the survival of one marine organism, ideally this research supports reasoning for the need of further research and regulations regarding the toxicity of cosmetics society uses on a daily basis. Currently within the United States, the governing body that controls cosmetics is the Food & Drug Administration (FDA), enacted by the Federal Food, Drug and Cosmetic Act of 1938 (US Food & Drug Administration, 2018). It is through this act that the FDA has set strict regulations regarding 11 types of cosmetic ingredients in its 80 years of existence. These ingredients include those that contain mercury, chloroform, and hexachlorophene, which are known to impact human health (US Food & Drug Administration, 2017).

As stated on the FDA website, "with the exception of color additives and a few prohibited ingredients, a cosmetic manufacturer may use almost any raw material as a cosmetic ingredient and market the product without an approval from FDA" (US Food & Drug Administration, 2018). In other words, as long as cosmetic ingredients and overall products are not a threat to human health, they can be manufactured without FDA approval. This leads cosmetic manufacturers to the freedom of being more self-regulated (Every CRS Report, 2012).

Since 1974, however, the FDA has developed the Voluntary Cosmetic Registration Program (VCRP), which encourages cosmetic manufacturers to register their facilities and ingredients in their products (Every CRS Report, 2012). As a consumer, however, it is difficult to know which facilities are associated with the VCRP. Consumer safety organizations like Environmental Working Group have pushed for a certification program for cosmetic manufacturers associated with VCRP as to notify consumers who is registered. However, the FDA has not yet approved of this motion (Every CRS Report, 2012). Increased registration for the VCRP, with the inclusion of a fee and certification program, could lead to more research, restrictions and funding toward understanding the environmental toxicological impacts cosmetic products in the United States market have today.

Alternatively, in other governing bodies such as the European Union, over 1,000 ingredients have been regulated or banned from cosmetics produced and sold through the EU Cosmetics Directive (Breast Cancer Prevention Partners, n.d.). Prior to the distribution or sale of cosmetics in the EU, products must undergo a pre-market safety assessment and registration process, thus providing for a stricter market of cosmetic products being sold (European Union, 2009). While neither the FDA nor the European Union heavily consider the environmental impact such ingredients have, the European Union at least emphasizes the need to thoroughly understand the effect each product could have on health as a whole.

An example of a country considering environment in its assessments and regulations instead is Australia. According to the Australian Academy of Science, every chemical ingredient that is imported, manufactured, and used must be scientifically evaluated for human and environmental health and approved before marketed by the National Industrial Chemicals Notifications and Assessment Scheme (NICNAS) or the Therapeutic Goods Administration (TGA) (Australian Academy of Science).

CONCLUSION

Through the findings of this research, as well as the discussion of how cosmetic policies stand within the United States, hopefully this Master's Project can contribute to the discussion of the importance of research behind cosmetic ingredients on impacts to marine environments. All tested ingredients and sunscreens indicate a major concern. The increased intensity of toxic effects from complex mixtures such as sunscreens, as evidenced by this research, shows a greater need for more studies and realistic assessments of environmental impacts from all cosmetics.

While there has been a rather strong focus on human health regarding sunscreens, little focus has been made on the environmental impact, except the recent studies behind sunscreen active ingredients. Based on how successful the oxybenzone studies have been from a science-to-policy path, it is recommended that more awareness discussions continue around the ingredients put on skin can have a connection with the natural processes of marine life. Ideally, this would lead to more human health and environmental research behind all cosmetic ingredients used in the United States.

From a public awareness standpoint, effective research translation is necessary to increase the knowledge-base of consumers and policy makers who would be concerned about impacts of cosmetic products. In addition to scientific articles being released on such toxicology research, scientists and professional communicators should take the additional step to effectively communicate implications of their research to the greater public. Considering the growing concern for the environment promoted by social media, short videos, PSAs, and infographics can lead to more informed consumers and policymakers. Increased public awareness and education behind consumer product ingredients can ideally lead to greater science-to-policy action, thus strengthening the regulations behind ingredient research and reducing the environmental impact of cosmetics used by tourists.

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