



The Effects of UV Filters on the Cnidarian Model Organism *Nematostella*

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CNIDARIAN IMMUNITY
LABORATORY

Introduction

- It is estimated that 4,000-6,000 tons of sunscreen are released into coral reef ecosystems each year and is contributing to the decline of global coral coverage throughout the world's oceans⁸.
- Some sunscreen formulations, like benzophenone-3 (BP-3), nanoparticle zinc oxide, and nanoparticle titanium dioxide, appear to have a negative effect on corals^{5, 7}.
- These UV filters have been shown to cause coral bleaching, zooxanthellae lysis, and morphological changes after as little as 8 hours of exposure in laboratory settings^{5, 7, 8}.
- The manner in which sunscreens alter coral's ability to regenerate and grow in the wild is not well studied.
- Nematostella* have incredible regenerative properties. After the foot of an individual is amputated, *Nematostella* can complete a full body regeneration, within 12-14 days^{1, 7}.
- Studying how the behavior, mortality rate, and regenerative process of *Nematostella* is altered by exposure to varying sunscreen formulations may provide important insight into how coral reefs are affected by UV filter pollution in the wild.

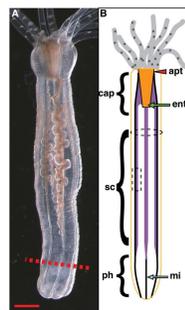


Figure 1: The anatomy of *Nematostella*¹.

Results

Initial Observations:				
	0% (Control)	1%	5%	10%
BP-3:	tentacles	normal	normal	tentacles retracted, body
Neutrogena:	retracted, still			condensed, still
Ultra Sheer				
Zinc Oxide:	tentacles	normal	normal	tentacles Extended,
Neutrogena:	retracted, still			moving body
Pure and Free				
Baby				
Titanium	normal	normal	normal	normal
Dioxide:				
Bare Republic				

Table 2: The recorded observations after the *Nematostella* were transferred to 12 well cell plate (0 hours). NOTE: Normal behavior is classified by the *Nematostella*'s tentacles being extended, the individual has a relaxed and soft body, and the animal responds to physical stimulus. If any of the *Nematostella* do not fit this criteria, it is considered abnormal behavior.

36.9 Hours:				
	0% (Control)	1%	5%	10%
BP-3:	normal	tentacles	dead	dead
Neutrogena:		retracted, no		
Ultra Sheer		response to		
Zinc Oxide:	normal	normal	normal	normal
Neutrogena:				
Pure and Free				
Baby				
Titanium	normal	normal	normal	normal
Dioxide:				
Bare Republic				

Table 3: The recorded observations after 36.9 hours of exposure to all 3 sunscreen brands.

84.9 Hours:				
	0% (Control)	1%	5%	10%
BP-3:	normal	tentacles and body	dead	dead, body
Neutrogena:		retracted, attempted to		appeared to be
Ultra Sheer		weakly stick to pipette,		partially
Zinc Oxide:	normal	body coated in layer of		dissolved and
Neutrogena:		sunscreen		tissue was
Pure and Free				opaquely white
Baby				
Titanium	normal	normal	normal	normal
Dioxide:				
Bare Republic				

Table 4: The recorded observations after 84.9 hours of exposure to all 3 sunscreen brands.

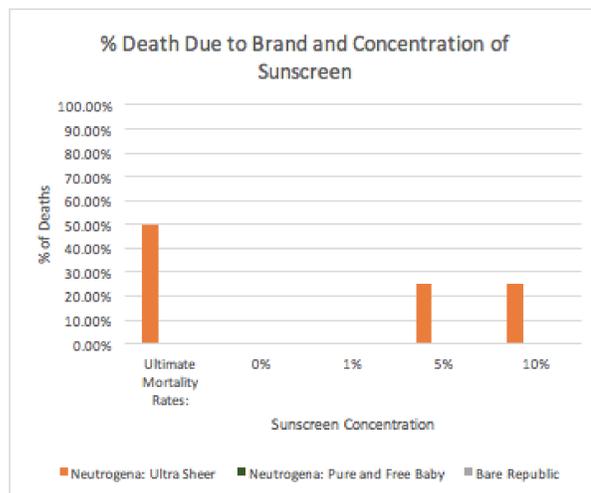


Figure 2: The percentage of mortality at the end of the 84.9 hour observation period for each brand of sunscreen at 0%, 1%, 5%, and 10% concentrations.

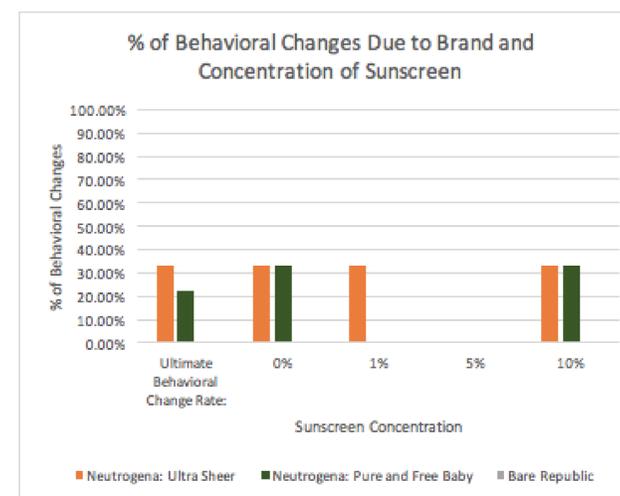


Figure 3: The percentage of behavioral changes recorded at the end of the 84.9 hour observation period for each brand of sunscreen at a 0%, 1%, 5%, and 10% concentration. NOTE: Normal behavior is classified by the *Nematostella*'s tentacles being extended, the individual has a relaxed and soft body, and the animal responds to physical stimulus. If any of the *Nematostella* do not fit this criteria, it is considered abnormal behavior.

Summary of Results

- As predicted, the BP-3 formulated sunscreen, Neutrogena Ultra Sheer, caused the highest rate of mortality within the pilot study. The overall mortality rate recorded over the 84.9 hour observation period was 50.0% (Fig. 2). This BP-3 formula was the only sunscreen that resulted in death.
- The Neutrogena Pure and Free Baby sunscreen, formulated with zinc oxide, resulted in an overall 22.22% change in behavior, with the most behavioral changes noted in the control and 10% conditions (Fig. 3).
- The Bare Republic Mineral sunscreen, formulated with titanium dioxide and zinc oxide, resulted in both a 0% mortality and 0% behavioral change rate (Fig. 2 and 3). This is the only sunscreen formula with no observable changes (Tables 2-4).
- Interestingly, the 10% solution of BP-3 appeared to have partially dissolved the body of the *Nematostella* after 84.9 hours (Table 4). The 10% BP-3 formulation was the only condition that caused cellular breakdown.

References

- Bossert et al., 2013
- Burton et al., 2009
- Corinaldesi et al., 2018
- Danovaro et al., 2003
- Danovaro et al., 2008
- DuBuc et al., 2014
- Downs et al., 2015
- Good, 2018
- Heron et al., 2016
- Ryan et al., 2006

Important Notes:

It is important to note that due to the COVID-19 pandemic, the entirety of this study was not completed. The goal of this experiment was to research how exposure to varying sunscreen formulations affected the regenerative process of post-amputated *Nematostella*, but due to unforeseen events, only the pilot study data that was collected and processed before RSMAS closed.

Discussion

- The partially dissolved *Nematostella* in the 10% concentration indicated that the BP-3 formula changed the morphology of the animals enough to break down the cellular structure at high concentrations, much like what has been previously observed in other cnidarians⁷.
- The zinc oxide and titanium dioxide formula observations do not align with previous research, as exposure to both nanoparticle zinc oxide and titanium dioxide caused altered behavior and high mortality rates in *Acropora spp.* corals due to bleaching in previous studies³.
- Previous work has suggested that nanoparticle zinc oxide and titanium dioxide formulas may cause mortality in corals due to the negative impact of these UV filters on the symbiotic zooxanthellae that live within the coral's tissues, and not the coral itself³. This may explain the differing results.
- The titanium dioxide formulation of the Bare Republic sunscreen appeared to be the most safe as there was both a 0% change in behavior and mortality rate, but more research must be conducted before conclusions are made (Fig. 1 and 2).

Future Research

- Although the amputation phase of this study was never completed, the pilot study data suggests potential parameters that should be investigated in the future.
- Homeodomain transcription factors, or homeoboxes, are developmental toolkits that have been conserved in *Nematostella* DNA and its evolutionary descendants. Because homeoboxes have been identified in both corals and *Nematostella*, the analysis of whether or not these genes are affected by UV filters and how they are affected may further reveal how sunscreens are altering cnidarian cell regeneration in the wild¹⁰.

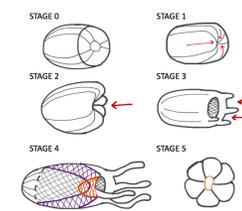


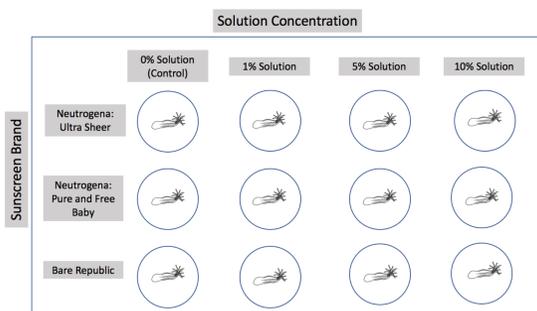
Figure 4: The 5 stage complete body regenerative process of *Nematostella*¹.

Acknowledgments

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Methods

- Individual *Nematostella* are each placed in one well in a 12 well cell culture dish according to figure below.



- Mortality rate, behavioral changes, and physical appearance were observed at 0 hrs, 36.9 hrs, and 84.9 hrs.

Brand Name	Active UV Filter Concentration
Neutrogena Ultra Sheer	Oxybenzone: 6%
Neutrogena Pure and Free Baby	Zinc Oxide: 21.6%
Bare Republic Mineral Sunscreen	Titanium Dioxide: 5.2% Zinc Oxide: 2.5%

Table 1: The brands of sunscreen used in this study and their active ingredients.