4-26-2019

The Effects of Ultraviolet Light Exposure on the Activity of Catalase in the Coelomocytes of Sea Urchins Lytechinus variegatus and Arbacia punctulata

Kandis Arlinghaus
karlinghaus01@bellarmine.edu

The Effects of Ultraviolet Light Exposure on the Activity of Catalase in the Coelomocytes of Sea Urchins *Lytechinus variegatus* and *Arbacia punctulata*

Kandis Arlinghaus

April 26, 2019

A Senior Honors Thesis Presented in Partial Fulfillment of the Requirements

of the Bellarmine University Honors Program

Advisor: Dr. Roberta Challener

Reader: Dr. Mary Huff

Reader: Dr. Steven Wilt

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

ABSTRACT

Many sea urchins play important ecological roles in their environments, and it is important to study the impacts of environmental stressors on their physiology. Ultraviolet radiation (UVR) exposure has significant negative impacts on marine organisms including an increase in reactive oxygen species (ROS). Oxidative damage by ROS at the cellular level can cause lipid peroxidation, DNA fragmentation, and even cell death that may result in inflammation or disease. To prevent this cellular damage, organisms generate enzymes, such as catalase, that breakdown ROS into harmless substances. Elevated catalase activities under UVB, a range of UVR from 280–315 nm, exposure have been detected for many aquatic organisms. Yet, it is unknown whether UVB exposure affects the activity of these antioxidant enzymes in many sea urchin species. *Lytechinus variegatus* is well known for its covering behavior in response to UVR exposure whereas *Arbacia punctulata* does not cover with any materials and remains fully exposed. Whether these behavioral differences are a result of differences in antioxidant enzyme activity in response to UVR exposure is not known. In this study, coelomocytes of *L. variegatus* and *A. punctulata* were exposed to UVB (302 nm) for one or two hours, and catalase activity was measured using colorimetric assays. Results suggest UVB exposure decreases catalase activity in the coelomocytes of *L. variegatus* ($p = < 0.0001$, t-test) and *A. punctulata* ($p = 0.0097$, t-test). Percent difference calculations found a greater percent decrease in catalase activity occurring in the coelomocytes of *L. variegatus* than in *A. punctulata* ($p = 0.0485$, t-test). Whether these observed differences in antioxidant activity are associated with covering behavior is yet to be determined.

INTRODUCTION

Sea Urchins in the Environment

Lytechinus variegatus and *Arbacia punctulata* are species of the phylum Echinodermata (**Figure 1**). Echinoderms are exclusively marine invertebrates with over 7,000 extant species that fall in to five classes. Of the five classes, this study is focused on the echinoids, i.e. sea urchins. Studying sea urchins is important because they are good models for measuring environmental stress and play important ecological roles in their environments. Many echinoderms are considered ecological keystone species because their presence is associated with greater species diversity and biomass.[1] This is due to their roles as both predator and prey; they stabilize habitats by controlling the species both above and below them in the food chain. If the keystone species is removed, the habitats tend to collapse. This collapse would likely cause other populations to experience negative ecological effects such as unstable community structures and lack of available niches.[2] The hypothesis of echinoderms as keystone species was tested by Robert Paine in the intertidal communities along the coast of Washington State. Paine tested his model with sea stars which are among the asteroid class of the echinoderm phylum. When Paine removed a predatory sea star from the community, a single species of mussel dominated the area which displaced several inferior species from the niche. When the predatory sea star was present, the sea stars consumed large numbers of mussels allowing more open niches for inferior competitors to utilize.[3] This keystone species example demonstrates why it is important to better understand echinoderms and how they function in their environments.

Figure 1. Covering behaviors of the sea urchins *Lytechinus variegatus* and *Arbacia punctulata.*

The sea urchins of interest in this study are *Lytechinus variegatus* and *Arbacia punctulata*. *L. variegatus* are found primarily located along the southeast coast of the United States, ranging from North Carolina to Santos, Brazil.[4] *L. variegatus* primarily inhabit sea grass beds in shallow waters of two to three meters and typically aggregate together in clusters that are advantageous for fertilization.[4] *L. variegatus* tend to conceal themselves with shells, grass, and other debris (**Figure 1**).[4] This covering behavior will be discussed further below in the subsection *Covering Behavior*. *L. variegatus* have important ecological roles in the structure of their ecosystems as they can be both predator and prey. *L. variegatus* primarily consumes seagrass and mussels and is prey to fish and several macroinvertebrates.[5] Studies by Valentine and Heck (1997, 2000) suggest *L. variegatus* play important ecological roles in controlling seasonal changes in seagrass abundance in some areas of the Florida Keys.[6, 7] Their results suggest both positive and negative effects of sea urchin grazing on seagrass abundance. For example, sea urchin grazing may stimulate seagrass biomass and production during the summer but may also lead to barren, unvegetated patches during fall and winter.

A. punctulata have a greater environmental range than *L. variegatus*, extending into colder temperatures of the northeast coast of the United States.[8] *A. punctulata* primarily inhabit sandy bottoms as well as rock jetties. This niche preference is due to their large suction discs located on their tube feet and greater clinging power that allows them to withstand the wave action of jetties. While *L. variegatus* are known for concealing themselves with materials, *A. punctulata* are not (**Figure 1**).[8] The ecological impacts of *A. punctulata* are not as well studied, and their effects on ecosystem structure is usually inferred from their diet. *Arbacia* consume a variety of food such as algae, sea grasses, sponges, coral polyps, dead fish, hydrozoans, sand dollars, mussels and bryozoans.[9] A study by Cobb and Lawrence (2005) suggests *A. punctulata* may change its feeding habitats based on the availability of algae.[10]

The sea urchins *Arbacia* and *Lytechinus* are able to distinguish different light sources and respond accordingly because they have photoreceptors in their tube feet.[8] Tube feet are a part of

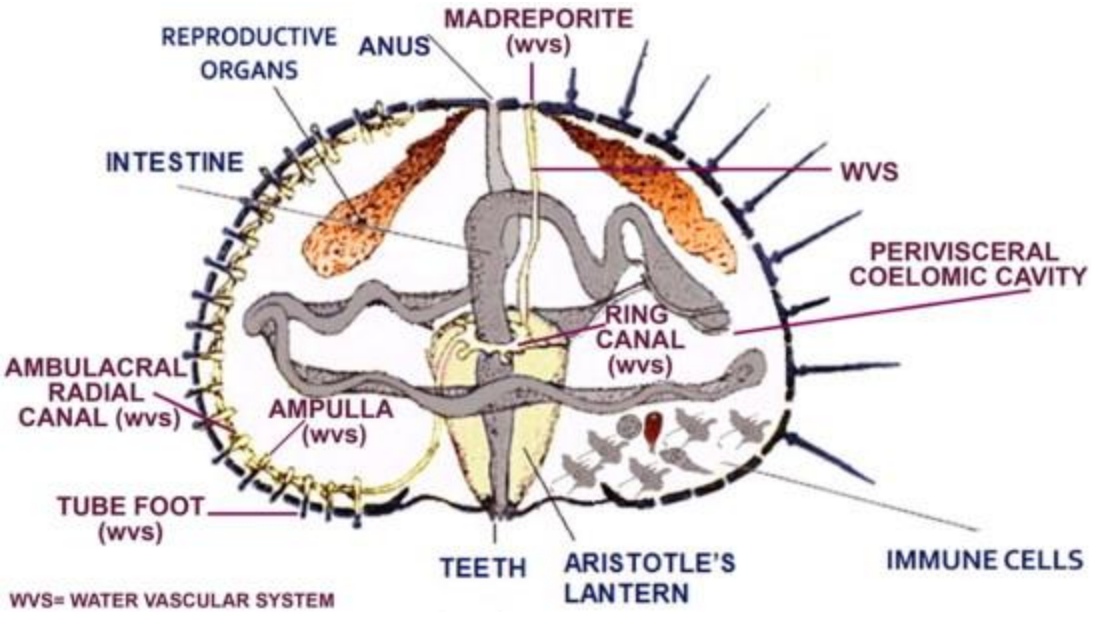

Figure 2. The basic anatomy of a sea urchin. Taken from Pinsino and Matranga (2015).[11]

the water vascular system, one of the defining features of the phylum (**Figure 2** in light yellow).[11] Water enters the system through the madreporite on the aboral surface. From there, water can flow through multiple canals to bring water to the tube feet. Tube feet are involved in movement, gas exchange, covering behavior, feeding and light detection. In addition to photoreceptors, genomic analyses of sea urchins have shown that olfactory receptors, sensory neurons, and opsins are expressed in the tube feet and react to chemical stimuli.[12] The water vascular system is also used for food and waste transportation and respiration.[13]

Sea Urchin Immunity

Immunology can be divided into two response types: innate and adaptive. The innate immune response is the nonspecific, first line of defense against pathogens while the adaptive immune response is the specific, secondary defense against infection and disease.[14] Examples of innate immune response would be bacteria-killing substances, membrane barriers, and scavenger cells. Examples of adaptive immune response would be B-cells, T-cells, and antibodies.[14] Both innate and adaptive immunity can be further divided into humoral and cellular components. Humoral immunity refers to the removal of foreign materials by the production of an agent that is transported by the circulatory system. In contrast, cellular immunity refers to the removal of foreign materials by cellular process such as phagocytosis or encapsulation.[15] Sea urchins and other echinoderms develop only innate immunity and not adaptive immunity, for innate immunity is an older evolutionary defense strategy.

Echinoderms have an open circulatory system that consists of the perivisceral coelomic system and water vascular system (**Figure 2**).[11, 15] Throughout the perivisceral coelomic cavity, circulating coelomic fluid has direct influence on all other internal cells in the tissues. Coelomic

fluid is involved with the transport of gases, nutrients and waste products between tissues.[15, 16] Coelomic fluid is composed of minerals and dissolved salts similar to sea water and contains lipids, proteins, sugars, and immune cells called coelomocytes.[2, 15] Six types of coelomocytes have been identified with the three most common consisting of amoebocytes, phagocytes, and vibratile cells (**Figure 3)**. Coelomocytes have been found in the tube feet, and therefore are potentially exposed to the external environmental conditions.[2, 17-19] They are important to the immunity because they use phagocytosis and encapsulation of the innate immune response to breakdown and remove foreign particles.[20] They also mediate a series of immune challenges by chemotaxis and secretion.[21] Coelomocytes are sensitive to environmental changes and are activated by stressors such as changes in temperature,[22, 23] acidification,[24] salinity,[25] UV radiation,[26] and pollutants[27] in experimentally controlled conditions. Additionally, field studies of sea urchins in environmentally stressful environments propose coelomocytes as novel cellular biosensors for ecotoxicological studies.[23, 28] Therefore, this easy manipulation, measurable response, and environmental sensitivity make coelomocytes good biosensors for monitoring environmental stress.[2, 11]

In the study by Branco et al. (2013), the sea urchins *Lytechinus variegatus* and *Echinometra lucunter* were exposed to three different temperatures for 1, 2, 7, and 14 days.[22] Coelomocytes were counted and assayed for phagocytic response, adhesion, and spreading. The results indicate that these two distinct species of sea urchins respond differently to rising sea temperature. *L. variegatus* seemed to be more susceptible to thermal stress, having more fluctuations in cell counts, phagocytosis, adhesion, and spreading. In contrast, the species *E. lucunter* presented changes only in cell counts at some temperatures and exposure periods and did not have changes in any of the other parameters measured. In another temperature dependent

study, Matranga et al. (2000) found that the coelomocytes in the sea urchin *Paracentrotus lividus* respond to temperature shock by the upregulation of the heat shock protein Hsp70.[23]

In a similar study by Figueiredo et al. (2016), the sea urchins *Lytechinus variegatus* and *Echinometra lucunter* were subjected to a pH of 8.0 (control group), 7.6 and 7.3.[24] Coelomocytes were analyzed for phagocytic capacity, phagocytic index, cell adhesion, and cell spreading. The pH of the coelomic fluid was also measured. The results suggest acidification affects the coelomic fluid pH and the amoebocyte phagocytic capacity in both species. However, the effects of a short-term exposure were reversible when the natural values were re-established.

A study examining the effects of salinity on sea urchin coelomocytes also demonstrated the usefulness of sea urchin coelomocytes as sentinels of environmental stress. Honorato et al. (2017) performed both *in vivo* and *in vitro* exposure experiments of the sea urchin *Echinometra lucunter*.[25] Phagocytic parameters (capacity and index), production of reactive oxygen species (ROS), mitochondrial activity, and ABC transporter activity were analyzed. The ABC transporter is a defense mechanism present in invertebrates which acts against physical and chemical stressors. The results suggest that changes in salinity did not affect the phagocytic capacity of coelomocytes but did affect ROS production and ABC transporter activity.

Of the several literature studies that have examined the effects of these environmental stressors on sea urchin coelomocytes, this study is focused on the effects of the harmful wavelengths of UVB (280–315 nm). Matranga et al. (2006) studied the effect of UVB radiation on sea urchin coelomocytes.[26] Their results showed a dose-dependent increase in the expression of heat shock protein 70 (Hsp70). Hsp70 is a stress marker protein in many organisms, and observation of an increase in Hsp70 is often indicative of increasing cellular stress. The increase of Hsp70 in coelomocytes exposed to UVB observed in the study by Matranga et al. (2006)

suggests UVB induces stress. Thus, it is important to further study how these cells respond to UVB. Furthermore, coelomocytes may be susceptible to UVB exposure and cellular damage since they are potentially exposed to the external environmental conditions through the tube feet.[19]

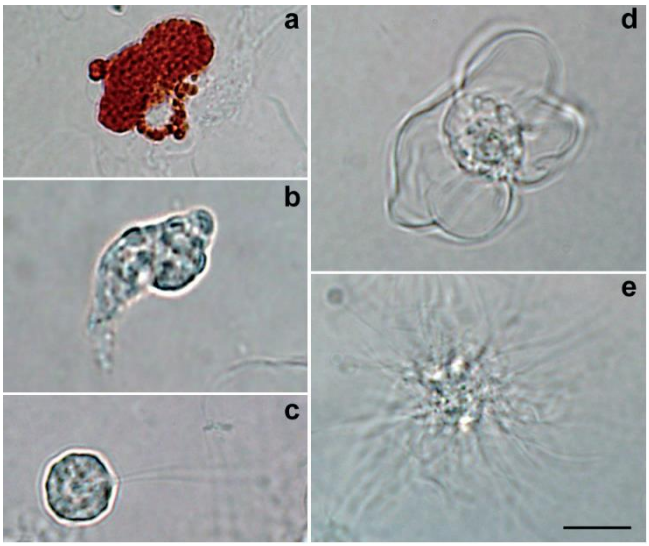

Figure 3. Morphologies of echinoderm coelomocytes. Taken from Matranga et al. (2005).[29]
A) red amoebocyte
B) colorless amoebocyte
C) vibratile cell
D) petaloid phagocyte
E) philopodial phagocyte
Bar 10 µm

Ultraviolet Radiation

The spectrum of light can be divided into seven sections based on energy. From lowest to highest energy they are: radio waves, microwaves, infrared radiation, visible light, ultraviolet, x-rays, and gamma rays. The ultraviolet (UV) region of the spectrum is 10 to 400 nm in wavelength. UV can be further subdivided into three regions based on wavelengths: UVA (315 – 400 nm), UVB (280–315 nm), and UVC (100–280 nm). UVA and UVB have larger wavelengths and reach the earth's surface unlike UVC which is absorbed by oxygen and ozone in the atmosphere and does not reach earth's surface. Although UVB has lower transmission to the earth's surface than UVA, UVB is predicted to increase at the earth's surface due to increasing ozone depletion.[30] From 1989 – 1993, UVB exposure at the surface has increased up to 35% in Canada.[30]

Upon entering the water column, UVB can penetrate depths up to 30 m even though the photons experience particulate absorption and scattering.[30] Echinoderms living in shallow coastal water are exposed to UVB on a daily basis. UVB effects in echinoderms are known to be controlled by biochemical and physiological processes (**Figure 4**).[1] The primary responses to

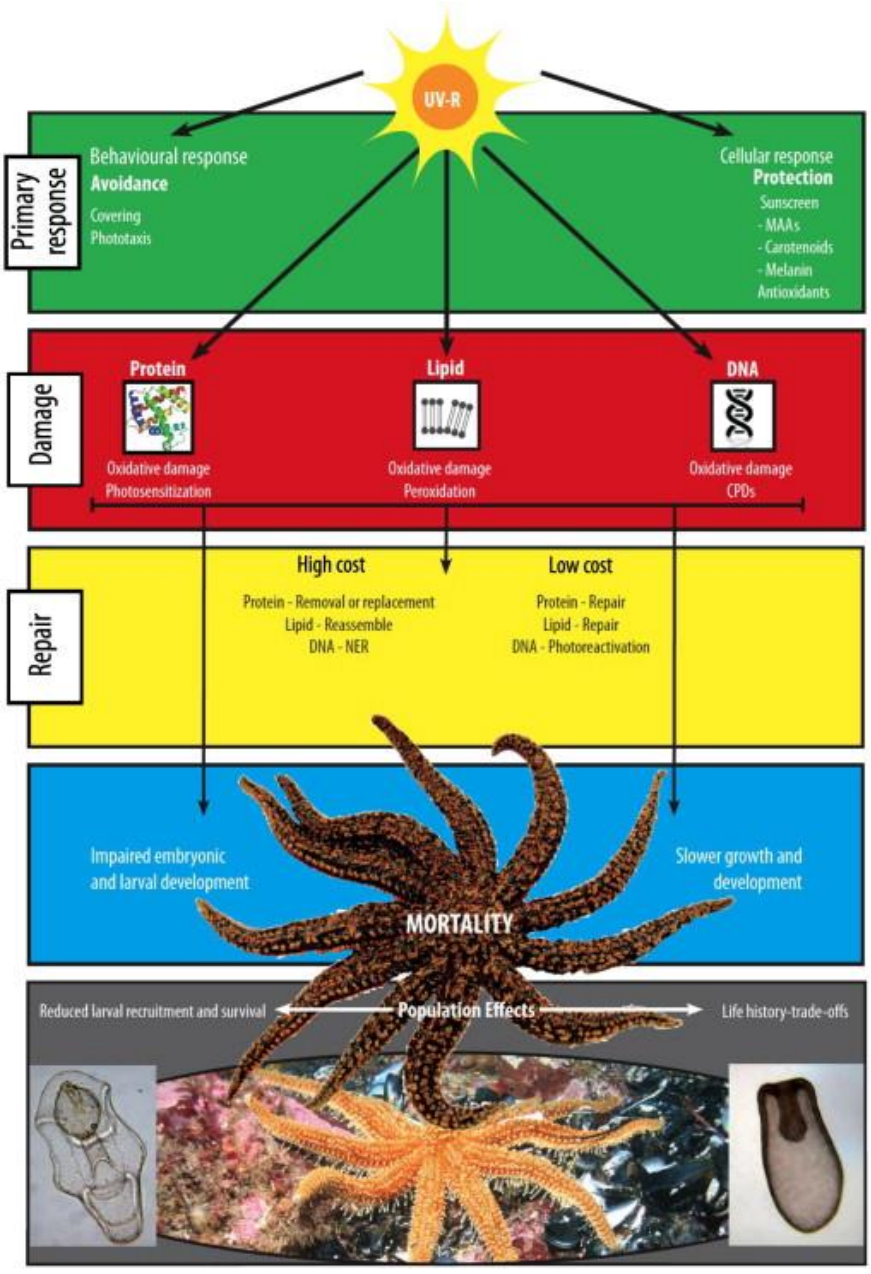

Figure 4. The effects of UVR on echinoderms. Taken from Lamare et al. (2011).[1]

UVB occur as both behavior and cellular responses. For example, sea urchins will use avoidance strategies such as covering and hiding in crevices to shield themselves from UVR.[1] Echinoderm cells contains biomolecular defenses against UVR exposure including mycosporine-like amino acids (MAAs), carotenoids, melanin, and antioxidants. MAAs are sunscreening molecules that have been shown to be capable of absorbing radiation in echinoderms by Adams and Shick (1996, 2001).[31, 32] Carotenoids are able to protect against UVR through antioxidation.[33] Melanin is another likely UV screening molecule and has been described in echinoderm species across the classes.[1] Antioxidants and antioxidant enzymes protect echinoderms from the oxidative stress that occurs as a result of UVR exposure.[1] Specifically, sea urchins contain a range of antioxidant compounds and antioxidant enzymes, such as catalase, which will be discussed below in the subsection *Oxidative Damage*. When these defensive strategies of MAAs, carotenoids, melanin, and antioxidants fail, echinoderms are more susceptible to an increased production of ROS when exposed to high levels of UVR.[1]

Covering Behavior

Behavioral responses to UVB, in particular covering behaviors, differ among various species of echinoderms.[34] These behavioral responses include avoiding light by hiding in crevices during the day and grazing during the night as well as using shells as shade on the aboral surface. Sea urchins are able to pick up items, such as shells, with tube feet and spines, and place them on their aboral surface. Covering behaviors in response to light, specifically to avoid ultraviolet light, have been described by Pawson and Pawson (2013) (references within).[35]

Sharp and Gray (1962) investigated the link between covering behavior and UVR in the sea urchins *Lytechinus variegatus*.[8] They found that covering occurred during the day, but the

material was dropped at night with specifically strong reactions to short wavelength UVR (UVA and UVB). Covering was also most frequent and complete during the spring when light intensity increases. Covering behavior increased in response to increased UVR exposure time. This suggests that UVR is a primary influence for covering in *L. variegatus*.[4, 36] Therefore, the threat of intensified UVR exposure may put evolutionary selection pressures on echinoderm populations based on the primary responses of covering behavior and cellular defense.[1] Adams (2001) found a similar covering response to UVR in the sea urchin *Strongylocentrotus droebachiensis.*[37] The results demonstrate that *Strongylocentrotus droebachiensis* seeks shade or covers itself in response to UVR. The covering reaction is greatest in response to UVB alone or to a combination of both UVA and UVB wavelengths. This greater sensitivity to UVB suggests that sea urchins are more sensitive to the shorter, more energetic wavelengths of UVB which cause more direct damage to cellular components.

In the same study by Sharp and Gray (1962), they also examined distribution differences among *L. variegatus* and *A. punctulata* in response to light.[8] Their results indicated that *L. variegatus* exhibit positive phototaxis to different light sources whereas *A. punctulata* exhibited negative phototaxis. Both species were also exposed to ultraviolet radiation (UVR). In experiments with UVR, *L. variegatus* and *A. punctulata* both demonstrated negative phototaxis. However, the species differed in their response time. *L. variegatus* had a quick negative response to UVR by moving away from the source and using shells for coverage. In contrast, *A. punctulata* had much slower response to UVR than *L. variegatus*. Therefore, according to Sharp and Gray (1962), *A. punctulata* can withstand a greater intensity of light for longer periods of time than *L. variegatus*.

The sea urchins *L. variegatus* and *A. punctulata* are of interest in this study because of these different shell covering strategies. These behaviors are considered to be responses to predation and UVR.[1] This relationship between UVR and avoidance strategies is interesting because not much is known about the different molecular and cellular responses, such as ROS production and oxidative damages, of sea urchin species with different covering behaviors.

While many studies suggest light and UVR exposure are the most common factors to induce a covering response, a debate still exists as to the reasons for this behavior. For example, a few deep-sea sea urchins living in the bathyal zone (200 - 2,000 m) exhibited this covering behavior even though UVR becomes attenuated when sunlight enters seawater, and essentially disappears by a depth of 20 m.[35] Therefore, further genetic research should be investigated to establish a heritable or evolutionary significant trait that can be attributed to covering behavior.

Oxidative Damage

ROS are free radicals containing oxygen, such as hydrogen peroxide (H_2O_2), that are produced by partial single electron reduction of oxygen.[1] These molecules are radicals because they contain at least one unpaired electron in the valence shell of the atomic nucleus. These molecules are highly reactive because they have a higher affinity to donate or accept another electron to achieve stability.[38] ROS are primarily produced as byproducts of the oxidative phosphorylation in mitochondrial respiration.[39] However, environmental stressors, such as UVR exposure, can increase the production of ROS.

When the primary defense of avoidance and cellular protection fails, damage can occur in proteins, lipids, and DNA. This impairment is caused by oxidative damage, photosensitization, peroxidation, and cyclobutene pyrimidine dimers (CPDs) (**Figure 4**).[1] Oxidative damage is

important to study because it is the most universal type of damage among these critical macromolecules. Oxidative damage occurs when reactive oxygen species (ROS) are over produced disrupting the cell's stable redox potential. This imbalance is known as oxidative stress.[38] Under normal conditions, cells combat ROS with antioxidants in order to maintain the cell's redox potential. However, when ROS increases due to environmental stressors, antioxidant defenses can no longer keep up with the buildup of these highly reactive intermediates in tissues.[1, 38] Oxidative damage occurs by many biological processes such as apoptosis, necrosis, and autophagy. Furthermore, extreme ROS production can destroy organelle structures and bio-molecules such as proteins, lipids, and DNA which can lead to an inflammatory response.[39]

Because ROS can disrupt cellular redox potential, studying the stressors that cause the chemical oxidation of oxygen molecules is important to stopping lethal damages to the cell and other bio-molecules. However, direct detection of ROS and other free radicals is difficult because they are highly reactive and short-lived. They also have nonspecific reactions; some ROS can diffuse across the cellular membrane and reduce a variety of molecules present in different organelles. Therefore, oxidative damage is more easily analyzed by measuring secondary molecules such as antioxidants and antioxidant enzymes.[38]

Antioxidants are reducing agents that inhibit oxidation. They prevent the interaction between radicals and biological targets to remove potentially damaging oxidizing agents such as ROS. Antioxidants are present in higher concentration in cells where radicals are being produced to reduce the potential for cellular damage. Antioxidant defense is just one of many defense mechanisms used to maintain cellular redox. Out of these various modes of protection, antioxidants are considered to be the most important because of its direct removal of ROS.[38] Antioxidants have also been shown to be a cellular response to UVR in echinoderms. Other

cellular protection responses in echinoderms include mycosporine-like amino acids (MAAs), carotenoids, and melanin (**Figure 4**).

Antioxidants can be enzymatic or non-enzymatic. The enzymatic antioxidant of interest in this study is catalase. Catalase is able to neutralize the reactive oxygen species hydrogen peroxide by decomposing it into molecular oxygen and water.[39] Catalase is a unique enzyme with a very low affinity for its substrate. Therefore, catalase can better remove hydrogen peroxide when it is present in high concentrations. Catalase can be found in various cellular organelles, including mitochondria and peroxisomes where hydrogen peroxide production is high.[40] In humans, catalase is present in most tissues and exists in different isozyme forms based on the function of the tissue.[40] The overall reaction for catalase cane be seen below.[38]

$$2H_2O_2 \xrightarrow{\text{catalase}} O_2 + 2H_2O$$

The purpose of this study was to investigate the effects of UVR exposure on the activity of the antioxidant enzyme catalase in the coelomocytes of two sea urchin species with different covering behaviors. The first objective was to establish standardized methods for housing organisms and decreasing variables that could affect cell concentrations. The second objective was to determine the effects of UVB on catalase activity in coelomocytes. The third objective was to compare these UVB effects between two sea urchin species with different covering behaviors. This study is important because little attention has been directed to the examination of UVR influences on mature echinoderm cells such as coelomocytes. In the course of a literature analysis review, published papers regarding the effects of UVR on ROS production and oxidative damage in coelomocytes were limited. Previous research by Du et al. (2013) demonstrated how antioxidant assays could determine oxidative damage in sea urchin

coelomocytes.[41] Specifically, catalase activity was measured using a colorimetric assay kit. However, no published papers comparing UVR effects on ROS production and oxidative damage in the echinoids *L. variegatus* and *A. punctulata* were identified. Investigating the different UVR-induced ROS outcomes of sea urchins with different covering behaviors will provide observations relating oxidative defenses and avoidance strategies.

MATERIALS AND METHODS

Dissection, Cell Count, and Dilution

Sea urchins were purchased from Gulf Specimen (Panacea, Florida) and delivered to Bellarmine University (Louisville, Kentucky) within 24 hours of departure. Organisms were housed in 5.5 and 10-gallon tanks with one organism in the 5.5-gallon tanks and two – three organisms in the 10-gallon tanks to ensure adequate water quality. Artificial seawater was made by adding artificial sea salt (Instant Ocean) to filtered deionized water. Tanks were prepared with 10 drops of ammonium chloride every other day for one – two weeks to accumulate healthy bacteria that would breakdown ammonia and nitrite when the sea urchins arrived. Upon arrival, sea urchins were briefly acclimated to the water temperature before immersion into the new tanks. Water salinity was adjusted to be most similar to the water shipped with the organisms. Salinity was then maintained at 27 – 29 ppt and measured using a multimeter (YSI).

Experiments were divided into two groups: Experiment 1 and Experiment 2. Three sea urchins of each species were used in Experiment 1 and dissected upon arrival. Ten sea urchins of each species were used in Experiment 2 and dissected after two weeks of acclimatization to the tanks. Sea urchin coelomic fluid was collected by making an individual cut in the peristomal membrane with scissors (**Figure 5**).[26] Volume of fluid was immediately measured and then

diluted with 1:1 Anticoagulant Solution (AS – 20mM Tris, 0.5M NaCl, 70mM EDTA, pH 7.2) following the methods of Arizza et al. (2007).[42] Diluted coelomic suspensions were stored in labelled glass storage test tubes at room temperature.

Figure 5. Individual cut in the peristomal membrane of the sea urchins *Lytechinus variegatus* and *Arbacia punctulata*. Cut is indicated by red arrow.

Cells were counted using a hemocytometer by diluting the cellular suspension with a 1:1 ratio of cells to trypan blue to indicate viable cells. The solution for cell counts was made by combining 20 µL of coelomic suspension with 20 µL of trypan blue in a microcentrifuge tube and was mixed by pipetting up and down. Counting was performed in triplicate with 10 µL of the solution pipetted onto the hemocytometer slide for each count. The outside boxes of the hematocytometer were counted and averaged to determine the number of viable cells per square and cell concentration (cells/mL). The coelomic solution was then diluted to a concentration of 2.0×10^5 cells/mL using AS following the methods of Matranga et al. (2006).[26]

Ultraviolet Radiation Exposure

Diluted coelomic suspensions were separated into experimental and control treatments. Experimental groups were exposed to UVB by a 302nm lamp (VWR) that was placed in a

container to block out other wavelengths in the electromagnetic spectrum. The lamp was placed six inches above the surface of the cell plates following the methods of Matranga et al. (2006).[26] The control plate was placed under a box to block out all wavelengths of light.

After exposure, three milliliters of diluted coelomic suspensions from each organism were aliquoted into separate petri dishes. The non-acclimatized sea urchins in Experiment 1 (n = 3 for each species) that were exposed to UVB for one hour while Experiment 2 used acclimatized organisms (n = 10 for each species) that were exposed to UVB for two hours. Immediately following one or two hours of UVB exposure, cells were incubated at room temperature in the dark for one hour. Control plates were incubated in the dark at room temperature for two hours (Experiment 1) or three hours (Experiment 2).

Cell Lysates

Following UVB exposure and incubation, cells lysates were prepared. After removing suspension, the plates were scraped with rubber cell scrapers. One milliliter of cold 1x Assay Buffer (prepared according to the manufacturer) from the Catalase Colorimetric kit (Invitrogen) was added to the plate. The lysate was collected by a micropipette and stored in microcentrifuge tubes. Solutions were centrifuged at 10,000 x g for 15 minutes at 4°C. Supernatant was collected and assayed immediately.

Antioxidant Enzyme Colorimetric Assays

Catalase activity was measured using a Catalase Colorimetric Activity Kit (Invitrogen) as seen in **Figure 6**. The kit was stored in the refrigerator at 4°C. Plates were read on a microplate reader (TECAN) with the software SPARK at 560 nm.

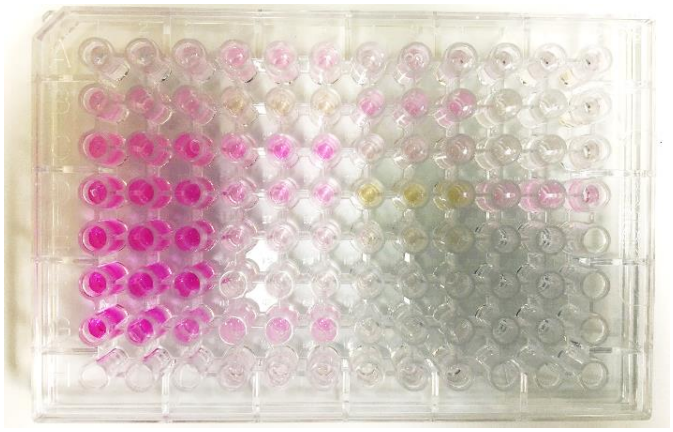

Figure 6. Colorimetric catalase activity microplate. The first three rows are the standard. Clear to light pink wells indicate high catalase activity while dark pink wells indicate low catalase activity.

Data Analysis

A standard curve was generated using a logarithmic trend line in Excel to calculate corresponding catalase activity. Data was analyzed using the computer software program PRISM. Analysis was performed using column data with two-tailed, paired t-tests.

RESULTS

The purpose of this study was to investigate the effects of UVR exposure on the activity of the antioxidant enzyme catalase in the coelomocytes of two sea urchin species with different covering behaviors. The first objective of this study was to establish standardized methods for housing organisms and decreasing variables that could affect cell concentrations. The second objective was to investigate catalase activity in coelomocytes after being exposed to UVB. This was measured by exposing coelomocytes to UVB for one or two hours and calculating activity using a colorimetric assay. The third objective of this study was to compare the catalase activity of two sea urchin species with different covering behaviors. This was standardized by performing a percent difference calculation to establish the percentage in which catalase activity changed for each species.

To determine the activity of catalase in coelomocytes, it was first important to establish standardized methods that limit variability. Therefore, housing methods and cellular concentrations were investigated to verify the importance of acclimatization on catalase activities. In Experiment 1, sea urchins were dissected upon arrival and were not acclimatized (**Figure 7**, dark green and dark purple). In Experiment 2, sea urchins were dissected following two weeks of acclimatization to the tanks (**Figure 7**, light green and light purple). Cells for both experiments were counted following dissection and the addition of 1:1 AS, so numbers do not represent original cell concentrations. Cell concentrations were higher in *L. variegatus* that were not acclimatized (**Figure 7**, dark green) than in *L. variegatus* that were acclimatized to the tanks for two weeks (**Figure 7**, light green). In contrast, cell concentrations were slightly lower in *A. punctulata* that were not acclimatized (**Figure 7**, dark purple) than in *A. punctulata* that were acclimatized to the tanks for two weeks (**Figure 7**, light purple). These data sets for the cell

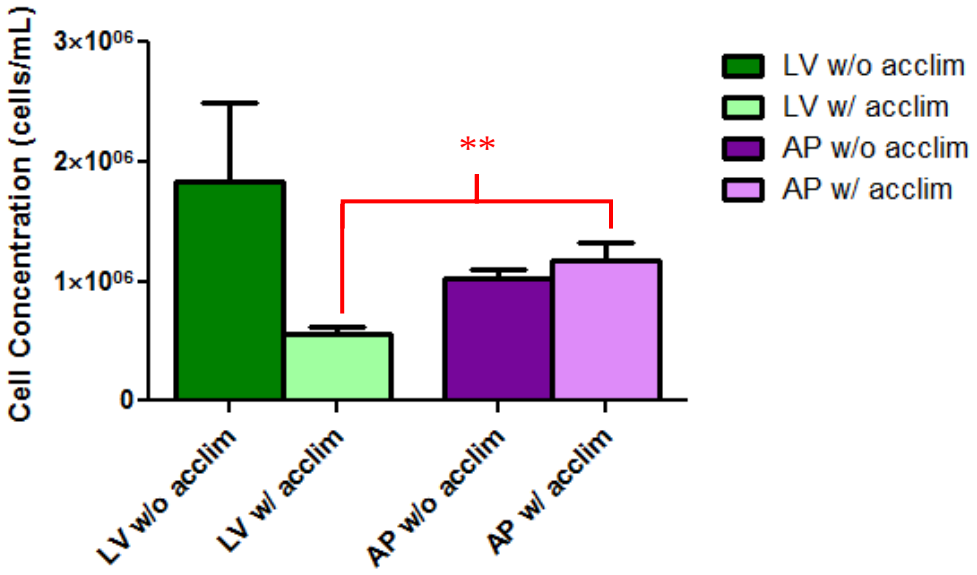

Figure 7. Cell concentrations in acclimatized and unacclimatized sea urchins. The sea urchins that were acclimatized to the tanks for 2 weeks did not have significantly different cell concentrations when compared to sea urchins that were dissected immediately after delivery. *L. variegatus* and *A. punctulata* had significantly different cell concentrations after acclimatization ($p = 0.0054$, t-test).

concentrations of both L. v*ariegatus* (p = 0.1666) and *A. punctulata* (p = 0.3914) with and without acclimatization were not significant. These insignificant data sets are mostly likely due to small sample size (n= 3 for no acclimatization). When comparing cell concentrations of both acclimatized species, the cell concentrations of *A. punctulata* (**Figure 7**, light purple) were higher than the cell concentrations of *L. variegatus* (**Figure 7**, light green). This data was significantly different (p = 0.0054, t-test).

To determine the effects of UVB light exposure on catalase activity, the catalase activity of UVB-exposed coelomocytes was measured and compared to the catalase activity of unexposed, control coelomocytes. Two experiments with different exposure times of one (Experiment 1, n = 3) or two (Experiment 2, n = 10) hours were performed. In Experiment 1, catalase activity was observed for both *L. variegatus* (p = 0.0585) and *A. punctulata* (p = 0.5981) coelomocytes exposed to UVB, this difference was not significant from the control treatments for either species. These results are most likely not significant because of the small sample size (n = 3) in Experiment 1.

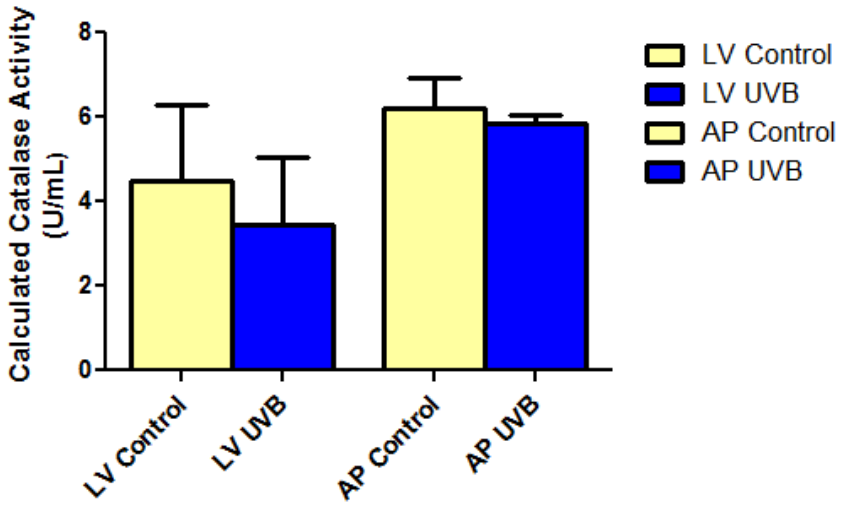

Figure 8. Catalase activity in coelomocytes exposed to UVB for 1 hour in both *Lytechinus variegatus* and *Arbacia punctulata*. There was no significant difference between experimental and control groups, and there was no significant difference between species for Experiment 1 (n = 3).

In Experiment 2, coelomocytes of each species were exposed to UVB for two hours, and the sample size for all treatments was increased (n = 10). This longer exposure period resulted in significant decrease in catalase activity for *L. variegatus* (p = <0.0001, t-test) and *A. punctulata* (p = 0.0097, t-test) shown in **Figure 9**.

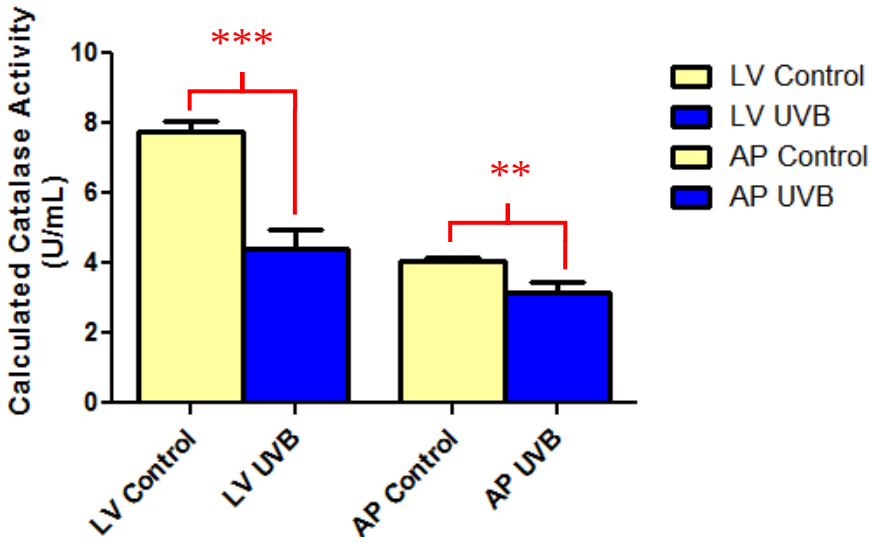

Figure 9. Catalase activity in coelomocytes exposed to UVB for 2 hours in both *Lytechinus variegatus* and *Arbacia punctulata*. Both species showed significant p-values for control vs. UVB catalase activity in Experiment 2 (n = 10). Catalase activity is given in units of U per mL such that one unit of catalase decomposes 1.0 µmol of H_2O_2 per minute at pH 7.0 and 25°C.
*** *L. variegatus* control vs. UVB: p = < 0.0001, t-test
** *A. punctulata* control vs. UVB: p = 0.0097, t-test

Percent difference was calculated to standardize the decrease of catalase activity relative to the control in order to compare the catalase activity of *L. variegatus* and *A. punctulata* after UVB exposure. The formula below was used to calculate percent difference of the UVB group (value 1) from the control group (value 2) for both Experiment 1 and Experiment 2.

$$\%Difference = \frac{|Value1 - Value2|}{\left[\frac{(Value1 + Value2)}{2}\right]} \times 100\%$$

For both Experiment 1(one-hour exposure) and Experiment 2 (two-hour exposure), *L. variegatus* had a greater decrease in catalase activity than *A. punctulata* (**Figure 10**). For Experiment 1, the percent decrease in the catalase activity after UVB exposure was not significantly different between species ($p = 0.2585$). Again, this data is most likely not significant because of the small sample size (n = 3). For Experiment 2, the percent decrease of catalase activity after UVB exposure was significantly different between species ($p = 0.0485$, t-test). *L. variegatus* had an average catalase activity decrease of 61% from the control, and *A. punctulata* had an average catalase activity decrease of 29% from the control (**Figure 10**). Therefore, *L. variegatus* had a significantly greater decrease in catalase activity than *A. punctulata*.

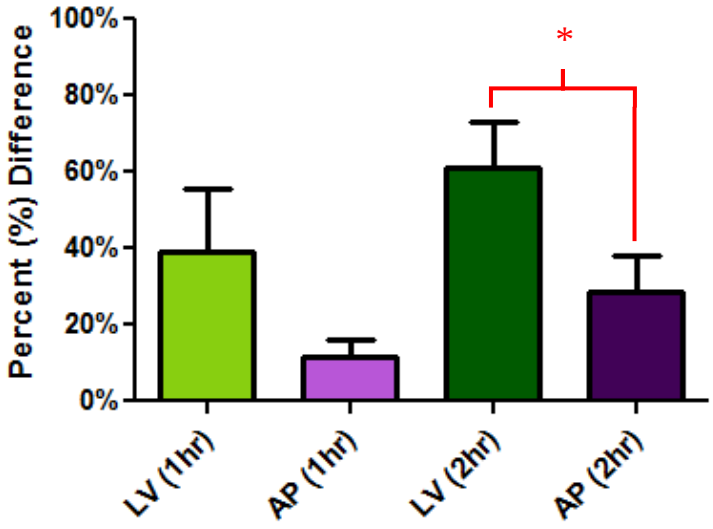

Figure 10. Percent difference in catalase activity in both 1 and 2 hour UVB exposed coelomocytes of *Lytechinus variegatus* and *Arbacia punctulata*. 2-hour exposure experiments had significant p-values for the percent difference of UVB catalase activity from the control catalase activity.
L. variegatus (1hr) vs. *A. punctulata* (1hr): $p = 0.2585$, t-test
* *L. variegatus* (2hr) vs. *A. punctulata* (2hr): $p = 0.0485$, t-test

DISCUSSION

This work describes the impacts of UVB exposure on the catalase enzyme in coelomocytes. Thus, the results of this study support the hypothesis that UVB affects the biochemical and physiological processes of coelomocytes and suggest enzyme differences may exist between echinoid species. The implications of the results presented above will be discussed in this section.

Sea Urchins in the Environment

This study serves as a model for the response of catalase to UVB in coelomocytes. Since coelomocytes are contained inside the body of sea urchins, coelomocytes would never be directly exposed to UV-B radiation in nature. Nevertheless, the findings of this study are relevant because coelomocytes are also located in other regions of the sea urchin which have contact to sunlight, and therefore, more indirect exposure to UVB. While coelomocytes are largely aggregated in the coelomic fluid, they are also found among other various tissues in sea urchins.[2] Specifically, coelomocytes aggregate in the coelomic epithelium which is a layer of the radial canal that lines the inside of tube feet.[17] It has also been reported that this coelomic epithelium is the site of coelomocyte production.[18] Furthermore, amebocytes were also found in the tube feet, gut wall, and other organs of the sea urchin.[19] Since coelomocytes are located in other regions of the sea urchins that are sensitive to UVB exposure (e.g., tube feet), coelomocytes are at risk for the resulting damages that can occur from UVB rays.

Acclimatization

An adjustment in methodology prompted the comparison of cell concentrations in acclimatized versus non-acclimatized sea urchins. Non-significant data for cell concentration

results suggest acclimatization did not greatly impact the cellular concentrations within the coelomic fluid of the sea urchins. Even though the results were not significant, it is still important to limit variables such as handling stress. Previous research indicated that handling stress and new environments can affect coelomocyte concentrations in sea urchins. Bertheussen and Seljelid (1978) reported that the coelomocyte concentration in the sea urchin *Strongylocentrotus droebachiensis* dropped significantly after the animal was kept in an aquarium for a period of time (exact time not mentioned).[43]

Handling stress is an important variable to consider when performing experiments with live animals because it not only impacts cellular concentrations but also affects molecular responses. In an experiment performed by Clow et al. (2000), molecular responses of sea urchin amoebocytes took several months to recover from handling stress.[44] In contrast, an investigation of amoebocytes ROS production in sea stars by Coteur et al. (2004) did not see an influence of handling stress on cellular activities.[45] It is possible that cellular activities such as amoebocyte concentration and ROS production are less sensitive to stress than other molecular responses.[45]

In studies of organisms outside the phylum Echinodermata, handling stress was shown to influence physiological responses. A study by Acerete et al. (2004) confirmed that magnitude and duration of physiological responses, such as hormone and glucose levels, are related to transport and acute handling of the fish *Perca fluviatilis*.[46] A study on rats by Capdevila et al. (2007) found that heart rate and activity levels were significantly changed after transportations.[47] Their results suggest rats need at least three days to acclimatize to a new environment. Handling stress and transports can affect many biological parameters of an organisms including cell concentrations, molecular responses, and physiological responses.[45-47] Therefore, it is important to use limited and consistent variables in experiments to decrease external influences.

Acclimatization is the most effective way to reduce handling stress and other environmental factors that could skew experimental results.

The cell concentration values reported in this study ranged between 2.9 x 10^5 cells/mL and 3.1 x 10^6 cells/mL. This range of sea urchin coelomocyte concentrations is supported by previous research (discussed below). However, it is first important to recognize that the proportion of each cell type and cell concentrations can vary among species (**Table 1**) and among organisms of the same species (**Table 2**).[15, 23] The variation among species is evident in the significant data between *L. variegatus* with acclimatization and *A. punctulata* with acclimatization (**Figure 7**). Despite the difference among species, previous studies from other sea urchin species found similar cell concentration ranges to this study, such as Matranga et al. (2000) who found *Paracentrotus lividus* coelomocyte concentrations between 1x10^6 and 2x10^6 cells per mL.[23] The variation among individuals of the same species is evident in a study investigating the sub populations of cells in the coelomic fluid of *Lytechinus variegatus* by

Table 1. Coelomocyte concentration of different echinoderm species. Taken from Chia and Xing (1996).[15]

Species	Coelomocyte concentration (10^6 cells/ml)	Reference
Cucumaria plankii	n.d.	Smith (1981)
Cucumaria miniata	60	Fontaine and Lambert (1977)
Eupentacta quinquesemita	60	Fontaine and Hall (1981)
Holothuria leucospilota	n.d.	Smith (1981)
Holothuria leucospilota	1.1 ± 0.4	Xing (unpublished data)
Holothuria polii	0.83	Canicatti et al. (1990)
Strongylocentrotus droebachiensis	5-10	Bertheussen and Seljelid (1978)
Strongylocentrotus purpuratus	n.d.	Smith et al. (1992)
Lytechinus pictus	4.25 ± 2.34	Laughlin (1989)
Arbacia puntulata	n.d.	Smith (1981)

Values are shown as mean ± S.D. when applicable.
n.d.: not determined.
n.a.: not applicable.

Borges et al. (2005).[48] In their study, they measured total coelomocyte concentrations collected from sea urchins kept with the mouth down and ones kept with the mouth up (**Table 2**). The high standard deviations of their results demonstrate how coelomocyte concentrations can vary even among organisms of the same species.

Table 2. Quantitative analysis of the coelomocytes/mm3 of perivisceral coelom of the sea urchin *Lytechinus variegatus* of the animals kept with the mouth down and of the animals kept with the mouth up. Taken from Borges et al. (2005).[48]

		Phagocytic Amoebocytes		Total Coelomocytes	
Position	Cell Type Region	Oral	Aboral	Oral	Aboral
Mouth down n=20	Average	2,016.00[1;]	1,270.50[1]	2,752.75	2,007.00
	S.D.	811.40	611.24	1,112.59	893.21
	%	73.24	63.30	100.00	100.00
Mouth up n=20	Average	2,375.25[1]	1,480.75[1]	3,061.50	2,192.50
	S.D.	898.70	792.90	920.64	1,008.55
	%	77.58	67.54	100.00	100.00

[1]Significant difference ($p < 0.05$) between oral and aboral.

Lytechinus variegatus cell concentrations reported in this study are compared to the *Lytechinus variegatus* cell concentration in the study by Borges et al. (2005) as shown in **Table 3**.[48] Average cell concentrations differ slightly but fall into the standard deviation range. Therefore, cell concentrations reported in this study are accurate and representative of previous literature.

Table 3. Comparison of *Lytechinus variegatus* cell concentrations between the results of Borges et al. (2005) and the results of this study.

Average Cell Concentrations (cells/mL)	
LV w/o acclimatization	3.66×10^6
LV w/ acclimatization	1.13×10^6
from Borges et al., (2005) – Oral	$2.753 \times 10^6 +/- 1.112 \times 10^6$
from Borges et al., (2005) – Aboral	$2.007 \times 10^6 +/- 8.932 \times 10^5$

Ultraviolet Radiation

Regardless of acclimatization, both the coelomocytes in Experiment 1 and Experiment 2 had decreased catalase concentrations after exposure to UVB. Furthermore, coelomocytes in Experiment 2 had a significant decrease in catalase activity for both *Lytechinus variegatus* and *Arbacia punctulata*. These results contrast embryonic sea urchin studies by Campanale et al. (2011) that found an increase in redox regulating enzymes when *Strongylocentrotus purpuratus* embryos were treated with UV.[49] The results of this study also contrast the findings of Black et al. (2011) that showed an increase in catalase mRNA expression in human corneal epithelial cells after UVB light treatment.[50]

This observed difference in catalase activity in response to UVB may be attributed to damaged protein structure, reversed enzymatic reactions, or over produced ROS. Decreased catalase activity in response to UVB exposure was seen in several other studies with beef, mouse, and rabbit tissue samples. Zigman et al. (1996) performed near-UV light experiments with purified beef liver catalase in which exposure to UV changed its physical state, inhibited its enzymatic activity, and altered its isoelectric point.[51] Enzyme analysis using polyacrylamide gel electrophoresis revealed a distinct band at 200 kDa, which was associated with UV exposure (**Figure 11**). These results by Zigman et al. (1996) suggest the decreased catalase activity observed in this study may be due structural changes caused by the direct UVR exposure to cells. Fuchs et al. (1989) found a similar decrease of catalase activity after UVB exposure in mice skin (**Figure 12**).[52] They suggest the decrease in catalase may be due to cellular catalase's sensitivity to visible light. Photooxidation of catalase is irreversible making the cell more susceptible to the damaging effects of ROS. Cejkova et al. (2000) also saw similar effects such that UVB rays decreased the activities of antioxidant enzymes including catalase in rabbit cornea.[53]

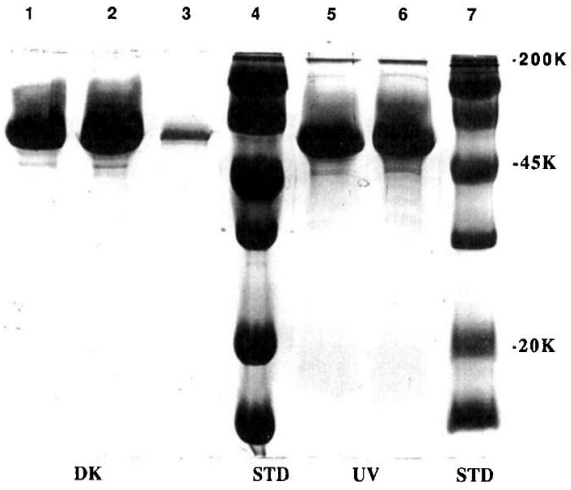

Figure 11. Polyacrylamide gel electrophoresis of purified beef liver catalase. Lanes 1 and 2 are for dark control catalase; lane 3 for unexposed purified catalase; lanes 4 and 7 are molecular weight protein standards; lanes 5 and 6 are UV-exposed samples.

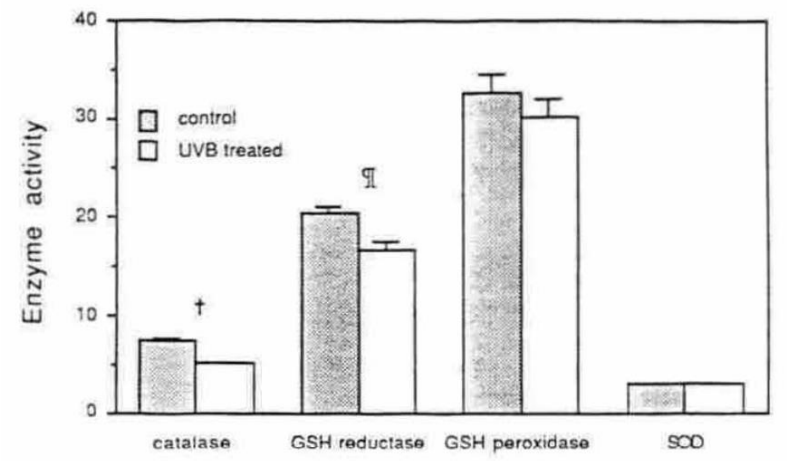

Figure 12. Enzyme activities in control and UVB treated skin. Catalase activity was measured in umol oxygen / mg protein / min. Catalase activity significantly decreased after UVB treatment in hairless mice skin. Taken from Fuchs et al. (1989).[52]

This decrease in catalase activity could also be explained by the peroxidatic properties of catalase. The forward reaction when catalase degrades hydrogen peroxide is known as a catalatic reaction. The reverse reaction when catalase oxidizes an oxygen containing electron donor, such as ethanol, is known as a peroxidatic reaction. Peroxidatic reactions occur when hydrogen peroxide concentrations are low.[54] A study by Heck et al. (2003) found that catalase in human

keratinocytes plays a direct role in generating oxidants in response to UVB light.[55] This means that instead of ridding the cell of ROS following UVB treatment, the enzyme facilitated ROS production. They suggest that this novel response of catalase is due to is peroxidatic properties. They hypothesize that, through the actions of catalase, high energy DNA damaging UVB light is absorbed by the enzyme and converted to reactive chemical intermediates that can then be detoxified by other cellular antioxidant enzymes. They also suggest that excessive ROS production by catalase may lead to oxidative stress, DNA damage, and the development of skin cancer.

Another possible explanation to this decreased catalase activity in sea urchin coelomocytes is the overabundance of ROS production by UVB. ROS can destroy both foreign particles and self-tissues since ROS are non-specific in their damage. They oxidize macromolecules such as lipids, proteins or nucleic acid resulting in lipid peroxidation, enzymatic activity disruption, and DNA damage.[56] Therefore, it is possible that ROS produced by UVB attacked self-macromolecules such as the catalase enzyme and disrupted its activity.[38] This self-degradation was the hypothesis behind the results of a study by Coteur et al. (2005) that looked at the impact of metals on ROS production *in vitro* and short-term *in vivo* sea urchin amoebocytes.[56] They found that exposure to metals caused an inhibition of the amoebocyte immune response.

Covering Behavior

After two hours of UVB exposure, *L. variegatus* had an average decrease in catalase activity of 61% from the control, and *A. punctulata* had a decrease in catalase activity of 29% from the control (**Figure 10**). These percent differences of UVB catalase activity from the control are significantly different between the two sea urchin species suggesting the cellular

activities of *L. variegatus* may be more sensitive to UVB than the cellular activities of *A. punctulata*. This difference in enzymatic response to UVB may be an evolutionary adaption of each species. The phylogenetic tree below depicts the class of echinoderms divided by genus (**Figure 13**). The dark lines within the boxed portion represent the genera of sea urchins that cover.

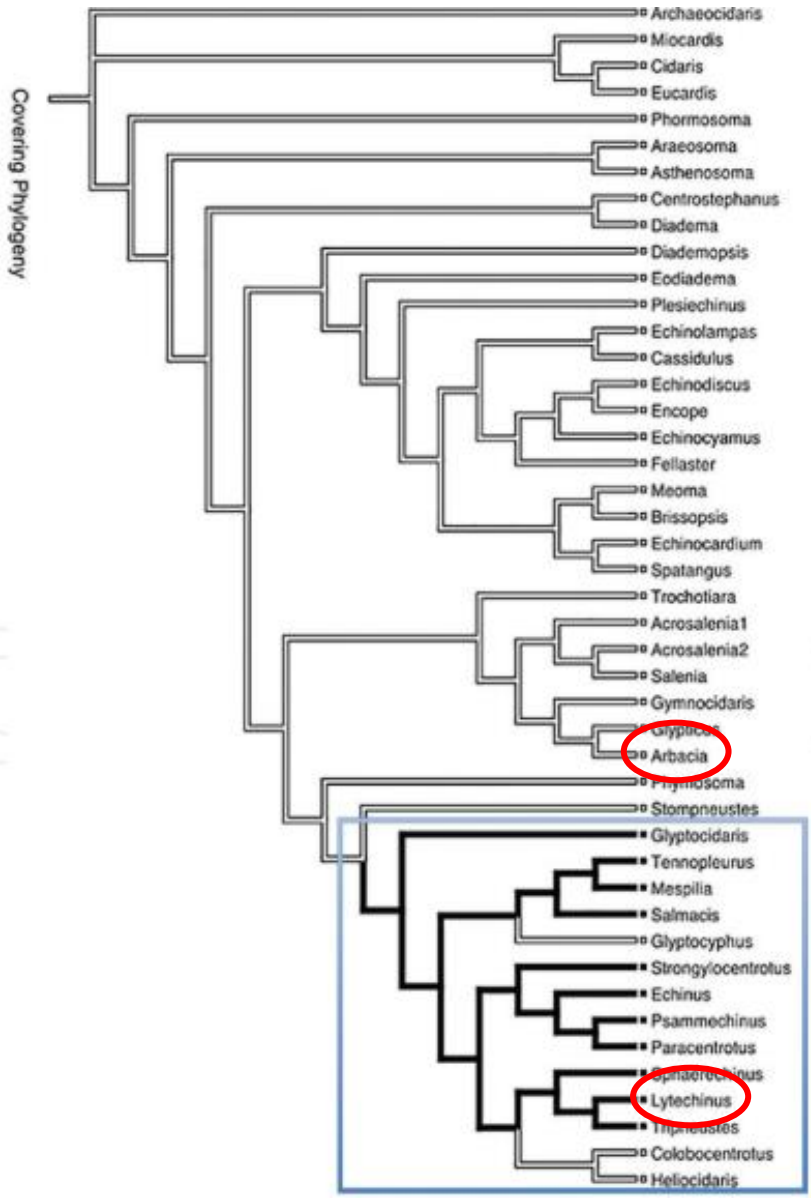

Figure 13. Sea urchin phylogeny. The species that cover are noted in dark lines within the box. Red circles indicate the genera *Lytechinus* and *Arbacia* and demonstrate the phylogenetic difference btw the two genera. Taken from Ziegenhorn (2017).[57]

A study by Ziegenhorn (2017) investigated the purpose of covering behavior between sea urchins of different genera.[57] Results from this experiment suggest that phylogenetic relationships provide a more predictive tool for determining the purpose of covering in an urchin genus than its environment. The four main reasons for covering in this study were predator defense, protection from mechanical damage (wave surge/floating debris), use as a food source, and protection from bright light (sunlight/UV). Protection from sunlight was the most common reason for using the behavior in the urchin genera considered, with six of the total fifteen genera covering primarily for this reason. Various other environmental factors were tested as predictive causes of covering behavior and were mapped on to the existing phylogenetic tree (**Figure 14**). The research from Zieganhorn (2017) presents a new hypothesis that there might be a relationship between the various uses of covering and other aspects of sea urchin biology, whether they be genetic or environmental considerations.[57] They propose that more closely

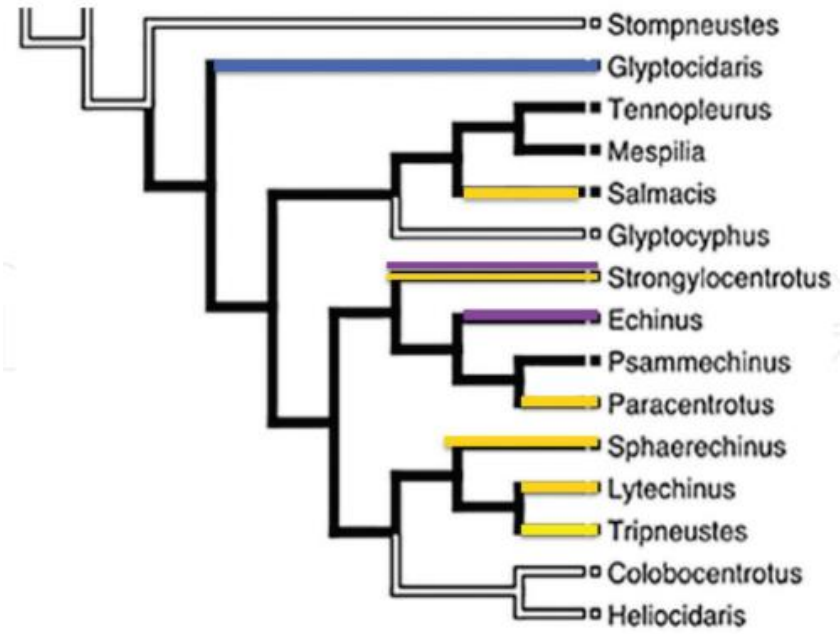

Figure 14. Covering behavior and evolutionary relationships. The colors represent the four investigated reasons for covering behavior: predator defense, mechanical defense (blue), food source (purple), and light protection (yellow). Taken from Ziegenhorn (2017).[57]

related sea urchin species cover for similar reasons. Therefore, predictions can be made for the cause of covering behavior of a species based on previous knowledge of phylogenetic relatives.

Ziegenhorn (2017) suggests that covering behavior may be genetically linked.[57] However, it is not known if these genetic relationships of defenses against environmental factors, such as UVR, can also be genetically linked to different cellular defenses among species. Tolerance to UVR differs among species and therefore some species are more likely to respond behaviorally to damaging UVR than others.[58] This relationship between covering behavior in response to UVR and antioxidant defenses among different species has not been previously studied in sea urchins. An investigation of the antioxidative enzymes in deep-sea fish by Janssens et al. (2000) suggests biochemical defenses against oxidative damage may have been an evolutionary adaptation.[59] This study supports that biochemical defenses like catalase may be an evolutionary adaption, and therefore, may be linked to the evolutionary split of covering behaviors in sea urchins.

Other cellular activities have been found to be related to covering behavior as a defense against UVB. A study by Kehas et al. (2005) found greater covering response in albino sea urchins suggesting a greater susceptibility to UV radiation.[60] They propose sea urchins can screen damaging radiation by pigment and UV-absorbing compounds such as mycosporine-like amino acids (MAAs). Photoprotection by MAAs is prevalent in several marine organisms in Antarctica where the ozone hole is the greatest.[58] A study on the pigmentation of *L. variegatus* by Millott (1956) found pale urchins tend to cover more quickly after being stripped of shells than dark ones.[61] Therefore, it is possible that pigmentation is a greater primary defense against UVR. Since *A. punctulata* is generally darker that *L. variegatus, A. punctulata* may need less coverage and less cellular activity to defend itself against UVR.

Whether these cellular differences are linked to the evolutionary genetics of covering behavior is uncertain. However, the enzymatic activity difference among *L. variegatus* and *A. punctulata* in response to UVB presented in this study indicate that a cellular difference exists among these two species. This cellular difference among sea urchin species may impact their distribution and abundance if UVB continues to increase due to ozone depletion. This change in distribution patterns may have profound effects on the ecosystems structure and function, possibly shifting communities towards more UV-tolerant species.[58]

Future Directions

Additional catalase colorimetric activity tests are needed to increase sample size and therefore increase the statistical significance of the data. UVB exposure on *in vivo* coelomocytes is essential to understand the relationship between UVB exposure in a more natural methodology to antioxidant enzymatic activity. Previous *in vivo* experiments of sea urchins exposed to UVB found significant negative effects on survival but did not analyze oxidative damage.[37, 62, 63] *In vivo* outcomes would validify the accuracy of *in vitro* results. Identification of ROS levels is essential to establish relationship between UVB exposure and ROS production. Measurement of other indicators of oxidative stress is also important in determining the relationship between defenses against UVR and covering behavior.

Furthermore, comprehensive assessments of UV-induced changes in proteomes can provide a deeper understanding of how UVB affects antioxidant enzymatic regulation. Measurements of the down regulation of catalase expression would enhance this experiment and can be performed with microarray which measures the relative abundance of mRNA for specific genes. Lastly, enzyme structure analysis is essential to assessing the hypothesis that the decreased catalase activity observed in this study was due to structural damage by UVB. Enzyme

crystallography could be us to determine if UVB damages the secondary or tertiary structure of catalase. DNA sequencing and protein NMR (nuclear magnetic resonance) could be performed to analyze the impacts of UVB on the primary structure of catalase.

ACKNOWLEDGEMENTS

I would like to thank Dr. Jon Blandford and the Bellarmine University Honors Program for all their support and encouragement throughout the completion of my Honors Senior Thesis. I would like to thank the Bellarmine University Biology Department for providing me with the funding, lab space, and equipment for this study to be performed. I would also like to thank the Bellarmine University Biochemistry and Molecular Biology Department for providing additional lab space and equipment. I would like to additionally thank the Student Government Association for awarding me a Student Government Association Undergraduate Research Grant for further funding. Also, I would like to thank Dr. Anne Bucalos for providing funding for this research project through a summer stipend. Furthermore, I would like to thank my committee members Dr. Mary Huff and Dr. Steven Wilt for their support and valuable feedback. Finally, I would like to thank my advisor Dr. Roberta Challener for her diligent guidance, expertise, and encouragement throughout this research project.

REFERENCES

[1] Lamare, M., Burritt, D., and Lister, K. (2011) Chapter Four - Ultraviolet Radiation and Echinoderms: Past, Present and Future Perspectives, In *Advances in Marine Biology* (Lesser, M., Ed.), pp 145-187, Academic Press.

[2] Smith, L. C., Ghosh, J., Buckley, K. M., Clow, L. A., Dheilly, N. M., Haug, T., Henson, J. H., Li, C., Lun, C. M., and Majeske, A. J. (2010) Echinoderm immunity, In *Invertebrate Immunity*, pp 260-301, Springer.

[3] Ricklefs, R., and Relyea, R. (2014) *Ecology: The Economy of Nature*, W.H. Freeman and Company, New York, NY.

[4] Moore, H. B., Jutare, T., Bauer, J., and Jones, J. (1963) The biology of Lytechinus variegatus, *Bulletin of Marine Science 13*, 23-53.

[5] Watts, S. A., McClintock, J. B., and Lawrence, J. M. (2007) Chapter 23 Ecology of Lytechinus, In *Developments in Aquaculture and Fisheries Science* (Lawrence, J. M., Ed.), pp 473-497, Elsevier.

[6] Valentine, J. F., Heck, K. L., Busby Jr, J., and Webb, D. (1997) Experimental evidence that herbivory increases shoot density and productivity in a subtropical turtlegrass (Thalassia testudinum) meadow, *Oecologia 112*, 193-200.

[7] Valentine, J. F., Heck Jr, K. L., Kirsch, K. D., and Webb, D. (2000) Role of sea urchin Lytechinus variegatus grazing in regulating subtropical turtlegrass Thalassia testudinum meadows in the Florida Keys (USA), *Marine Ecology Progress Series 200*, 213-228.

[8] Sharp, D. T., and Gray, I. E. (1962) Studies on Factors Affecting the Local Distribution of Two Sea Urchins, Arbacia Punctulata and Lytechinus Variegatus, *Ecology 43*, 309-313.

[9] Gianguzza, P., and Bonaviri, C. (2013) Chapter 19 - Arbacia, In *Developments in Aquaculture and Fisheries Science* (Lawrence, J. M., Ed.), pp 275-283, Elsevier.

[10] Cobb, J., and Lawrence, J. M. (2005) Diets and coexistence of the sea urchins Lytechinus variegatus and Arbacia punctulata (Echinodermata) along the central Florida gulf coast, *Marine Ecology Progress Series 295*, 171-182.

[11] Pinsino, A., and Matranga, V. (2015) Sea urchin immune cells as sentinels of environmental stress, *Developmental & Comparative Immunology 49*, 198-205.

[12] Raible, F., Tessmar-Raible, K., Arboleda, E., Kaller, T., Bork, P., Arendt, D., and Arnone, M. I. (2006) Opsins and clusters of sensory G-protein-coupled receptors in the sea urchin genome, *Developmental Biology 300*, 461-475.

[13] Lawrence, J. M. (1987) *A Functional Biology of Echinoderms*, Johns Hopkins University Press.

[14] Vivier, E., Raulet, D. H., Moretta, A., Caligiuri, M. A., Zitvogel, L., Lanier, L. L., Yokoyama, W. M., and Ugolini, S. (2011) Innate or Adaptive Immunity? The Example of Natural Killer Cells, *Science 331*, 44-49.

[15] Chia, F.-S., and Xing, J. (1996) Echinoderm coelomocytes, *ZOOLOGICAL STUDIES-TAIPEI- 35*, 231-254.

[16] Ziegler, A., Schröder, L., Ogurreck, M., Faber, C., and Stach, T. (2012) Evolution of a novel muscle design in sea urchins (Echinodermata: Echinoidea), *PLoS One 7*, e37520.

[17] Nichols, D. (1959) The histology and activities of the tube-feet of Echinocyamus pusillus, *Journal of Cell Science 3*, 539-555.

[18] Pinsino, A., Thorndyke, M. C., and Matranga, V. (2007) Coelomocytes and post-traumatic response in the common sea star Asterias rubens, *Cell stress & chaperones 12*, 331-341.

[19] Mangiaterra, M. B., and Silva, J. R. (2001) Induced inflammatory process in the sea urchin Lytechinus variegatus, *Invertebrate Biology 120*, 178-184.

[20] Smith, L. C., Rast, J. P., Brockton, V., Terwilliger, D. P., Nair, S. V., Buckley, K. M., and Majeske, A. J. (2006) The sea urchin immune system, *Invertebrate Survival Journal 3*, 25-39.

[21] Gross, P. S., Al-Sharif, W. Z., Clow, L. A., and Smith, L. C. (1999) Echinoderm immunity and the evolution of the complement system, *Developmental & Comparative Immunology 23*, 429-442.

[22] Branco, P. C., Borges, J. C. S., Santos, M. F., Jensch Junior, B. E., and da Silva, J. R. M. C. (2013) The impact of rising sea temperature on innate immune parameters in the tropical subtidal sea urchin Lytechinus variegatus and the intertidal sea urchin Echinometra lucunter, *Marine Environmental Research 92*, 95-101.

[23] Matranga, V., Toia, G., Bonaventura, R., and Müller, W. E. G. (2000) Cellular and biochemical responses to environmental and experimentally induced stress in sea urchin coelomocytes, *Cell Stress & Chaperones 5*, 113-120.

[24] Leite Figueiredo, D. A., Branco, P. C., dos Santos, D. A., Emerenciano, A. K., Iunes, R. S., Shimada Borges, J. C., and Machado Cunha da Silva, J. R. (2016) Ocean acidification affects parameters of immune response and extracellular pH in tropical sea urchins Lytechinus variegatus and Echinometra luccunter, *Aquatic Toxicology 180*, 84-94.

[25] Honorato, T. B. M., Boni, R., da Silva, P. M., and Marques-Santos, L. F. (2017) Effects of salinity on the immune system cells of the tropical sea urchin Echinometra lucunter, *Journal of Experimental Marine Biology and Ecology 486*, 22-31.

[26] Matranga, V., Pinsino, A., Celi, M., Bella, G. D., and Natoli, A. (2006) Impacts of UV-B radiation on short-term cultures of sea urchin coelomocytes, *Marine Biology 149*, 25-34.

[27] Coteur, G., Danis, B., Fowler, S. W., Teyssié, J. L., Dubois, P., and Warnau, M. (2001) Effects of PCBs on Reactive Oxygen Species (ROS) Production by the Immune Cells of Paracentrotus lividus (Echinodermata), *Marine Pollution Bulletin 42*, 667-672.

[28] Pinsino, A., Della Torre, C., Sammarini, V., Bonaventura, R., Amato, E., and Matranga, V. (2008) Sea urchin coelomocytes as a novel cellular biosensor of environmental stress: a field study in the Tremiti Island Marine Protected Area, Southern Adriatic Sea, Italy, *Cell Biology and Toxicology 24*, 541-552.

[29] Matranga, V., Pinsino, A., Celi, M., Natoli, A., Bonaventura, R., Schröder, H., and Müller, W. (2005) Monitoring chemical and physical stress using sea urchin immune cells, In *Echinodermata*, pp 85-110, Springer.

[30] De Mora, S. J., Demers, S., and Vernet, M. (2000) *The Effects of UV Radiation in the Marine Environment*, Cambridge University Press, Cambridge.

[31] Adams, N. L., and Shick, J. M. (1996) Mycosporine-like amino acids provide protection against ultraviolet radiation in eggs of the green sea urchin Strongylocentrotus droebachiensis, *Photochemistry and Photobiology 64*, 149-158.

[32] Adams, N., and Shick, J. (2001) Mycosporine-like amino acids prevent UVB-induced abnormalities during early development of the green sea urchin Strongylocentrotus droebachiensis, *Marine Biology 138*, 267-280.

[33] Rastogi, R. P., Sinha, R. P., Singh, S. P., and Häder, D.-P. (2010) Photoprotective compounds from marine organisms, *Journal of industrial microbiology & biotechnology 37*, 537-558.

[34] Lawrence, J. M. (1976) Covering response in sea urchins, *Nature 262*, 490-491.

[35] Pawson, D. L., and Pawson, D. J. (2013) Bathyal sea urchins of the Bahamas, with notes on covering behavior in deep sea echinoids (Echinodermata: Echinoidea), *Deep Sea Research Part II: Topical Studies in Oceanography 92*, 207-213.

[36] Sigg, J. E., Lloyd-Knight, K. M., and Boal, J. G. (2007) UV radiation influences covering behaviour in the urchin Lytechinus variegatus, *Journal of the Marine Biological Association of the United Kingdom 87*, 1257-1261.

[37] Adams, N. L. (2001) UV radiation evokes negative phototaxis and covering behavior in the sea urchin Strongylocentrotus droebachiensis, *Marine Ecology Progress Series 213*, 87-95.

[38] Kohen, R., and Nyska, A. (2002) Invited Review: Oxidation of Biological Systems: Oxidative Stress Phenomena, Antioxidants, Redox Reactions, and Methods for Their Quantification, *Toxicologic Pathology 30*, 620-650.

[39] He, L., He, T., Farrar, S., Ji, L., Liu, T., and Ma, X. (2017) Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species, *Cellular Physiology and Biochemistry 44*, 532-553.

[40] Tehrani, H. S., and Moosavi-Movahedi, A. A. (2018) Catalase and its mysteries, *Progress in Biophysics and Molecular Biology 140*, 5-12.

[41] Du, C., Anderson, A., Lortie, M., Parsons, R., and Bodnar, A. (2013) Oxidative damage and cellular defense mechanisms in sea urchin models of aging, *Free Radical Biology and Medicine 63*, 254-263.

[42] Arizza, V., Giaramita, F. T., Parrinello, D., Cammarata, M., and Parrinello, N. (2007) Cell cooperation in coelomocyte cytotoxic activity of Paracentrotus lividus coelomocytes, *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 147*, 389-394.

[43] Bertheussen, K., and Seljelid, R. (1978) Echinoid phagocytes in vitro, *Experimental cell research 111*, 401-412.

[44] Clow, L. A., Gross, P. S., Shih, C.-S., and Smith, L. C. (2000) Expression of SpC3, the sea urchin complement component, in response to lipopolysaccharide, *Immunogenetics 51*, 1021-1033.

[45] Coteur, G., Corriere, N., and Dubois, P. (2004) Environmental factors influencing the immune responses of the common European starfish (Asterias rubens), *Fish & Shellfish Immunology 16*, 51-63.

[46] Acerete, L., Balasch, J., Espinosa, E., Josa, A., and Tort, L. (2004) Physiological responses in Eurasian perch (Perca fluviatilis, L.) subjected to stress by transport and handling, *Aquaculture 237*, 167-178.

[47] Capdevila, S., Giral, M., Ruiz de La Torre, J., Russell, R., and Kramer, K. (2007) Acclimatization of rats after ground transportation to a new animal facility, *Laboratory Animals 41*, 255-261.

[48] Borges, J. C. S., Jensch-Junior, B. E., Garrido, P. A. G., Mangiaterra, M. B. B. C. D., and Silva, J. R. M. C. (2005) Phagocytic amoebocyte sub populations in the perivisceral coelom of the sea urchin Lytechinus variegatus (Lamarck, 1816), *Journal of Experimental Zoology Part A: Comparative Experimental Biology 303A*, 241-248.

[49] Campanale, J. P., Tomanek, L., and Adams, N. L. (2011) Exposure to ultraviolet radiation causes proteomic changes in embryos of the purple sea urchin, Strongylocentrotus purpuratus, *Journal of experimental marine biology and ecology 397*, 106-120.

[50] Black, A. T., Gordon, M. K., Heck, D. E., Gallo, M. A., Laskin, D. L., and Laskin, J. D. (2011) UVB light regulates expression of antioxidants and inflammatory mediators in human corneal epithelial cells, *Biochemical pharmacology 81*, 873-880.

[51] Zigman, S., Reddan, J., Schultz, J. B., and McDaniel, T. (1996) Structural and functional changes in catalase induced by near-UV radiation, *Photochemistry and photobiology 63*, 818-824.

[52] Fuchs, J., Huflejt, M. E., Rothfuss, L. M., Wilson, D. S., Carcamo, G., and Packer, L. (1989) Impairment of enzymic and nonenzymic antioxidants in skin by UVB irradiation, *Journal of investigative dermatology 93*, 769-773.

[53] Cejková, J., Stipek, S., Crkovska, J., and Ardan, T. (2000) Changes of superoxide dismutase, catalase and glutathione peroxidase in the corneal epithelium after UVB rays. Histochemical and biochemical study, *Histology and histopathology 15*, 1043-1050.

[54] Oshino, N., Oshino, R., and Chance, B. (1973) The characteristics of the "peroxidatic" reaction of catalase in ethanol oxidation, *The Biochemical journal 131*, 555-563.

[55] Heck, D. E., Vetrano, A. M., Mariano, T. M., and Laskin, J. D. (2003) UVB light stimulates production of reactive oxygen species unexpected role for catalase, *Journal of Biological Chemistry 278*, 22432-22436.

[56] Coteur, G., Danis, B., and Dubois, P. (2005) Echinoderm reactive oxygen species (ROS) production measured by peroxidase, luminol-enhanced chemiluminescence (PLCL) as an immunotoxicological tool, In *Echinodermata*, pp 71-83, Springer.

[57] Ziegenhorn, M. A. (2017) Sea Urchin Covering Behavior: A Comparative Review, In *Sea Urchin-From Environment to Aquaculture and Biomedicine*, InTech.

[58] Leech, D. M., and Johnsen, S. (2003) Behavioral responses–UVR avoidance and vision, *UV effects in aquatic organisms and ecosystems. Royal Society of Chemistry, Cambridge*, 455-481.

[59] Janssens, B. J., Childress, J. J., Baguet, F., and Rees, J.-F. (2000) Reduced enzymatic antioxidative defense in deep-sea fish, *Journal of Experimental Biology 203*, 3717-3725.

[60] Kehas, A. J., Theoharides, K. A., and Gilbert, J. J. (2005) Effect of sunlight intensity and albinism on the covering response of the Caribbean sea urchin Tripneustes ventricosus, *Marine Biology 146*, 1111-1117.

[61] Millott, N. (1956) The Covering Reaction of Sea-Urchins: I. A Preliminary Account Of Covering in the Tropical Echinoid Lytechinus Variegatus (Lamarck), and its Relation to Light, *Journal of experimental biology 33*, 508-523.

[62] Zhao, C., Zhang, L., Shi, D., Chi, X., Yin, D., Sun, J., Ding, J., Yang, M., and Chang, Y. (2018) Carryover effects of short-term UV-B radiation on fitness related traits of the sea urchin Strongylocentrotus intermedius, *Ecotoxicology and Environmental Safety 164*, 659-664.

[63] Lees, D. C., and Carter, G. A. (1972) The Covering Response to Surge, Sunlight, and Ultraviolet Light in Lytechinus Anamesus (Echinoidea), *Ecology 53*, 1127-1133.