

Parental Dietary β -Carotene Intake in *Lytechinus variegatus* Affects Early Development of Offspring Exposed to UV Radiation

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ABSTRACT

Planktonic embryos of *Lytechinus variegatus* utilize photo-protective mechanisms to minimize the deleterious effects of solar ultraviolet radiation (UVR) exposure. It was hypothesized that embryos from parents who received supplemental β -carotene in their diets would be more resistant to the effects of UVR than embryos from parents who received no supplement. Adult *Lytechinus variegatus* broodstock with gonads in the growing phase were fed diets either with or without supplemental β -carotene for 5 months and subsequently induced to spawn. Fertilized eggs were collected from each feed treatment and exposed to differing intensities of UVA (0-4 J/m²) or UVB (0-100 mJ/cm²) radiation. Larval mortality counts and developmental status were recorded at 34 and 55 hours post-fertilization and compared between feed treatments. Embryos derived from sea urchins consuming supplemental β -carotene developed at a slower rate than those whose parents did not consume supplemental β -carotene. Increasing intensities of UVA and UVB radiation were positively correlated with larval mortality in both dietary treatments. UVB induced higher mortality than UVA. Larval mortality in the β -carotene supplemented treatment was significantly higher than the non-supplemented treatment. These data suggest that dietary supplements of carotenoid do not provide photo-protection and may enhance, through unknown mechanisms, the deleterious effects of UV exposure.

Lytechinus variegatus are broadcast spawners, releasing gametes directly into the water column. In shallow water, eggs and developing embryos can be subject to the detrimental effects of ultraviolet radiation (UVR), which in many embryos can negatively impact survivorship, delay development, and promote genetic degradation (Lesser and Barry, 2003; Campanale et al., 2011). UVR can penetrate several meters into coastal waters (Booth and Morrow, 1997). The most biologically deleterious portion of the ultraviolet wavelength is classified as ultraviolet-B (UVB) radiation, which spans the electromagnetic spectra between 280 and 320 nanometers. UVB radiation exposure delays development and produces abnormalities in sea urchin embryos (Adams and Schick, 2001), thought to be the result of DNA damage. Additionally, UV induced protein damage interferes with normal cellular processes and lipid damage disrupts cell membranes

(Bancroft et al. 2007). While many organisms can repair UV induced cellular damage, the efficiency of these processes differ among species (Blaustein et al., 1994). Ultraviolet-A radiation (UVA), spanning between 320 and 400 nm, can form reactive oxidant species (ROS) in irradiated cellular constituents *in vitro* (McCormick et al., 1976, Czocharlska et al., 1984; Vile and Tyrrell, 1994). Oxygen free radicals generated from UVB or UVA exposure can lead to point mutations and deletions in DNA (de Gruijl. 2002). Embryos of the sea urchin *Strongylocentrotus droebachiensis* have displayed sensitivity to UVA and UVB radiation (Lesser et al. 2006). UVR has been shown to impact covering behavior and larval settlement in *Lytechinus variegatus* (Sigg et al. 2007, Tauchman and Pomory 2011).

In a study comparing UVR sensitivity of the embryos of three urchin species Lesser et al. (2006) reported that embryos with low concentrations of UV absorbing compounds were more sensitive to UVR and endured more frequent occurrences of cyclopyrimidine dimers. There is evidence suggesting that maternally derived UV-protective compounds are obtained in the diet and sequestered in developing ova (Carroll and Shick, 1996). Examples of these compounds include microsporine-like amino acids, (MAA's) and carotenoids. MAA's have been shown to increase UV protection and prevent UV induced developmental abnormalities in the eggs of the sea urchin *Strongylocentrotus droebachiensis* (Adams and Shick, 1996; Adams and Shick, 2001). We hypothesize that these compounds function similarly in *Lytechinus variegatus*. Carotenoids and MAA's are secondary metabolites that function as molecular sunscreens in some marine organisms that live in environments with high intensities of sunlight. In addition, it is suggested that carotenoids within the embryo may function as antioxidants or photo-protectants (Lamare and Hoffman, 2004). Bottom feeders such as sea urchins are presumably shielded from the majority of harmful UVR, however their gametes and embryos are subject to upwelling currents which may increase their exposure to UVR near the water's surface. Sea urchins presumably obtain some carotenoids through the diet; however, the carotenoid echinenone is suggested to be synthesized within the urchin gut wall as well as the ovary (Tsushima et al., 1993). Nutrients from the maternal diet are sequestered in developing ova, which facilitate embryonic development post-fertilization (Herrera, 1995). This nutritive compliment includes carotenoids (Griffiths, 1966). Consequently, sea urchin embryos must solely rely on maternally derived stores of nutrition until they reach the feeding pluteus stage.

This study compares the effects of parental dietary carotenoid intake on the development and survival of early embryos exposed to either indoor fluorescent lighting (control) or to differing levels of indoor UVA or UVB radiation. Fertilized eggs from two diet treatments were exposed to environmentally relevant levels of UVA or UVB radiation.

MATERIALS AND METHODS

Sample Collection

Lytechinus variegatus (ca. 8 to 32g) were collected from the subtidal zone of St. Joseph's Bay (30°N, 85.5°W), FL and transported in 75-liter aerated coolers to the University of Alabama at Birmingham. Sea urchins were placed into multiple 75-liter aquaria plumbed into an artificial saltwater recirculating system with biological filtration and starved for 1 month to standardize their nutritional status (Spirlet et al. 2000). Water conditions were maintained as follows: 32 ± 0.5 ppt

salinity, $24 \pm 1^\circ\text{C}$, D.O. 7 ± 2 ppm, ammonia <0.2 ppm, nitrite <0.2 ppm, nitrate <80 ppm, and pH 8.2.

Raceway design and water quality

The experiment was performed in a large raceway (235 cm x 53 cm x 31 cm, L x W x H, as described by Taylor 2006). A 160 x 23 cm (L x H) center baffle in the center of the raceway allowed for recirculating water flow by an in-line utility pump (Supreme[®] Mag Drive Utility Pump, 700 gallons of water/hour). The utility pump removed saltwater from the raceway on one side of the baffle. Water was then passed through a mechanical and biological filter and returned to the raceway on the opposite side of the baffle. The flow rate of the resulting current was approximately $9.7 - 12.6 \text{ cm s}^{-1}$. Water was continually pumped through a Life Guard Aquatics Ultraviolet Sterilizer Model QL-15. Water conditions were maintained as follows: 32 ± 0.5 ppt salinity, $22 \pm 2^\circ\text{C}$, D.O. 7 ± 2 ppm, ammonia <0.2 ppm, nitrite <0.2 ppm, nitrate <80 ppm, and pH 8.2. Nutrients were measured using the API Saltwater Master Liquid Test Kit. A 12-hour dark, 12-hour light photoperiod was maintained. Feces were siphoned 3 times per week.

Twenty-four sea urchins were randomly divided among six plastic mesh cages (ca. 23 cm x 23 cm x 14 cm high, with 5 mm open mesh sides and bottom) within the raceway ($n = 2 - 5$ urchins per cage). The floor of each cage was elevated above the bottom of the raceway with PVC spacers (ca. 4 cm) to allow unimpeded water circulation. Cages were rotated within the raceway each week to prevent bias due to cage position. Urchins in three cages were fed a formulated diet (C-) without supplemental carotenoids, and urchins in the other three were fed the same formulated diet supplemented with carotenoids (C+). A fluorescent light fixture was mounted above the center of the raceway, and it was fitted with 2 bulbs emitting blue actinic light (450-500 nm peak spectral output). In a previous study, urchins exposed primarily to blue actinic light for over 12 weeks developed gonads that were redder in color than urchins that were not (Taylor et al., 2014). We hypothesized that urchins exposed to actinic light would sequester higher levels of carotenoids into their gonads and transfer them to developing ova. The light fixture was provided by Nova Extreme 91.44 cm, 2 * 39 Watt T5 systems. Actinic bulbs were supplied by Giesemann 39 watt Power Chrome Actinic + fluorescent bulbs. According to Geiseman, these bulbs may emit a small amount of UVA radiation; however, the peak emission for these bulbs is between 450 and 500 nm. Each parental treatment was exposed to the same levels of actinic and possible UVA spectra throughout the feeding period. The raceway was fitted with an enclosure made from opaque plastic sheeting and suspended from supports 70 cm high around the top of the raceway to obstruct outside light from entering. The enclosure was vented at the top to allow heat dissipation. Lux was measured daily with a Milwaukee MW700 waterproof probe in the raceway from 3 equally spaced locations along a central line beneath the lighting system. A lux reading of 2400 ± 200 lux at the center measuring site was maintained by raising or lowering the light ballasts. Lux levels were maintained to insure that any possible carotenoid metabolism or deposition in the gonad due to light intensity or quality was kept consistent in the parents (Taylor et al., 2014).

Feed and feed preparation

Two formulated feeds were produced using both purified and semi-purified ingredients. One feed was supplemented with a commercial carotenoid preparation to a final weight per volume of 1.7%

(MP Biomedicals) containing β -carotene and xanthophylls (C+), and the other was not supplemented with carotenoids (C-, replaced with diatomaceous earth). The carotenoid concentration selected for this study had been shown to optimize gonad color between yellow and orange in previous experiments (Taylor et al., 2014). Previous experiments show no negative growth outcomes due to this carotenoid inclusion in previous experiments with adults. All other nutrients remained constant between diets including any ingredients that may have contained microsporine-like amino acids (not tested). Dry ingredients were mixed with a Hobart stand mixer (Model A-200, Hobart Corporation, Troy, OH) and blended for 40 minutes. Liquid ingredients were added, and the mixture was blended for an additional 10 minutes to a mash-like consistency. The feed was extruded using a meat chopper attachment (Model A-200, Hobart Corporation, Troy, OH) fitted with a 4.8 mm die. Feed strands were separated and dried on wire trays for 48 hours. Final moisture content of all feed treatments was 8–10%. Feed was stored in air-tight storage bags at 4°C until used. Feed proffered to urchins was kept in opaque and air tight containers in order to prevent degradation of β -carotene within the urchin diet.

Within the raceway, 12 sea urchins were proffered a daily *ad libitum* ration of the diet supplemented with carotenoids (C+). The remaining 12 were proffered an *ad libitum* ration of the diet that lacked supplemental carotenoids (C-).

Spawning and fertilization

After 5 months (May – October 2012) of feeding, sea urchins from each feed treatment were induced to spawn via injection of 1 mL 0.1 M acetylcholine through the peristomial membrane. Gametes from each feed treatment were collected and diluted with synthetic saltwater (ASW, Crystal Sea salt). Sperm from 2 males of each treatment was diluted by adding 2-3 drops of sperm into 1 L of ASW, inducing activation, and individuals were then combined. This protocol has been shown previously to optimize fertilization success in previous studies (Moseley, 2009). Eggs from 2 females from each treatment were mixed within each treatment and diluted with 1 L ASW. Eggs were fertilized with 3 mL dilute sperm solution from 2 males in the same treatment. One mL of media containing fertilized eggs from each treatment was transferred to 30 mL glass Petri plates and labeled according to UVR exposure or as control (exposed to fluorescent room light only). Successful fertilization was determined by visualization of a raised fertilization envelope within 5 min. Control fertilization was always >95%.

UVR exposure and data acquisition

Two replicates of fertilized eggs from each treatment were exposed to one of 6 levels of UVB radiation (10, 20, 40, 60, 80 or 100 mJ/cm²) or 3 levels of UVA radiation (1, 2, or 4 J/m²) using a Daavlin, UVA/UVB Research Irradiation Unit (Bryan, OH). This device was programmed to irradiate samples at predefined levels of UVA or UVB. Three replicates of controls for each broodstock feed received no UVR exposure. Exposures were conducted in glass Petri dishes with the tops removed during the exposure period. Embryos were incubated at 24-26° C.

Development of each embryonic treatment was assessed at 6.5 and 34 hours post fertilization (HPF). Embryos were sorted into developmental categories which were chosen after an initial survey of the population but before counting. Survival was recorded at 34 and 55 HPF. The

counter was blinded to each treatment assessed for development and survival. Evaluations were recorded after removing a 1 mL subsample containing embryos from each replicate Petri dish and transferring it to a clean Petri dish. The mean n value for 6.5 hrs was 167 (range: 24-286). The mean n value for 34 hrs was 97 (range: 63-101) and the mean n for 55 hrs was 100 (range: 94-102). Counts at each diet treatment and exposure level were combined among replicate Petri dishes and compared for each of 3 assessment periods. Samples from each Petri dish were preserved in 50% Davidson's solution (40% formalin, 220 ml; glacial acetic acid, 115 ml; 95% ethanol, 330 ml; distilled water, 335 ml) at each counting period and were photographed for developmental stage identification. Developmental stage categories were compared between diet treatments within a UVR exposure level.

Statistics

Data from each replicate were pooled in this study. Differences in survival or development within and between diet treatments were calculated using a 2x2 contingency table with a Yates corrected Chi-Sq. 2-tailed p value. Significance was determined at $p < 0.05$.

RESULTS

Development at 6.5 HPF

Since embryos were not moving at this time period, survival could not be accurately determined. Exposure to as little as 1 J/m² UVA (Fig. 1) and 20 mJ/cm² UVB (Fig. 2) altered development within both diet treatments. For treatments that received no UVR, C- (no carotenoid supplementation) produced higher numbers of early blastula and mature blastula than C+(supplemented with carotenoids) ($p < 0.001$, 0.001 respectively). At all levels of UVA exposure (1, 2, and 4 J/m²), C- produced higher numbers of blastula than C+ ($p < 0.001$, 0.001, 0.001, respectively) (Fig. 1). No significant differences in development were found between diet treatments at 10 mJ/cm² UVB exposure. Significantly higher numbers of normal blastula were recorded in C+ at UVB exposures of 20 and 40 mJ/cm² ($p < 0.001$, 0.001, respectively). A significant majority of embryos in C+ at 60 mJ/cm² were pre-blastula (Fig. 2). A majority of embryos from both diet treatments at exposure levels of 80 and 100 mJ/cm² were abnormally developed at the preblastula stage and were not counted at this stage (Fig. 2).

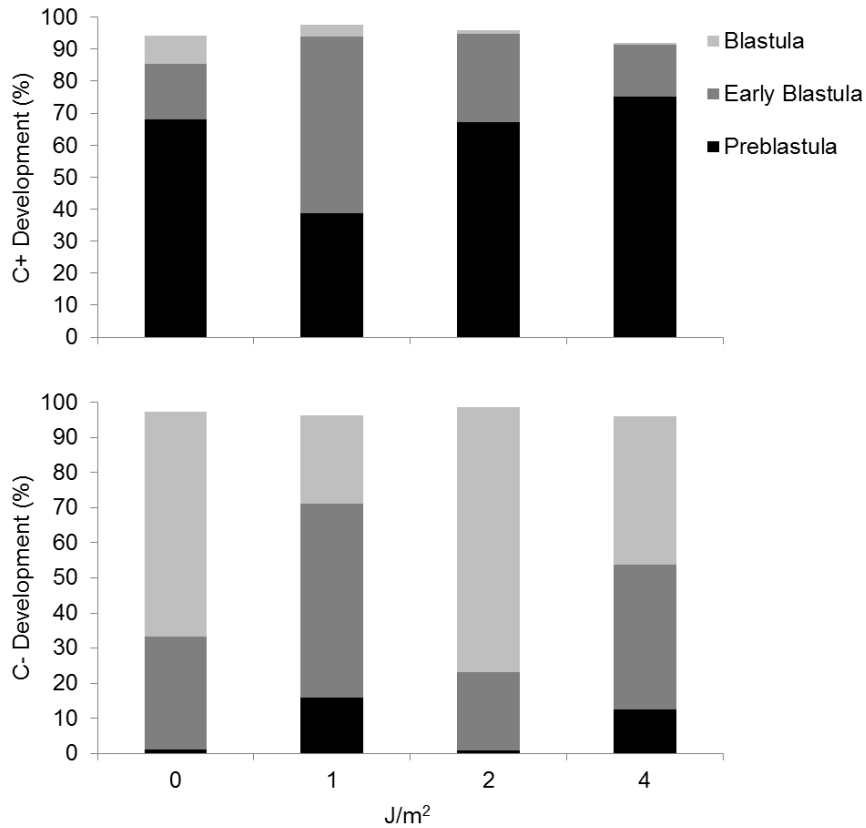


Figure 1. Percent of *Lytechinus variegatus* embryos (6.5 HPF) at each developmental stage in which the parents were fed a diet (A) with or (B) without supplemental β -carotene. Embryos were exposed to one of 3 intensities of UVA radiation at 1 HPF. Exposure to as little as 1 J/m² UVA resulted in significant alteration in development within both diet treatments. At all levels of UVA exposure (1, 2, and 4 J/m²) there were significantly higher numbers of blastula in C-.

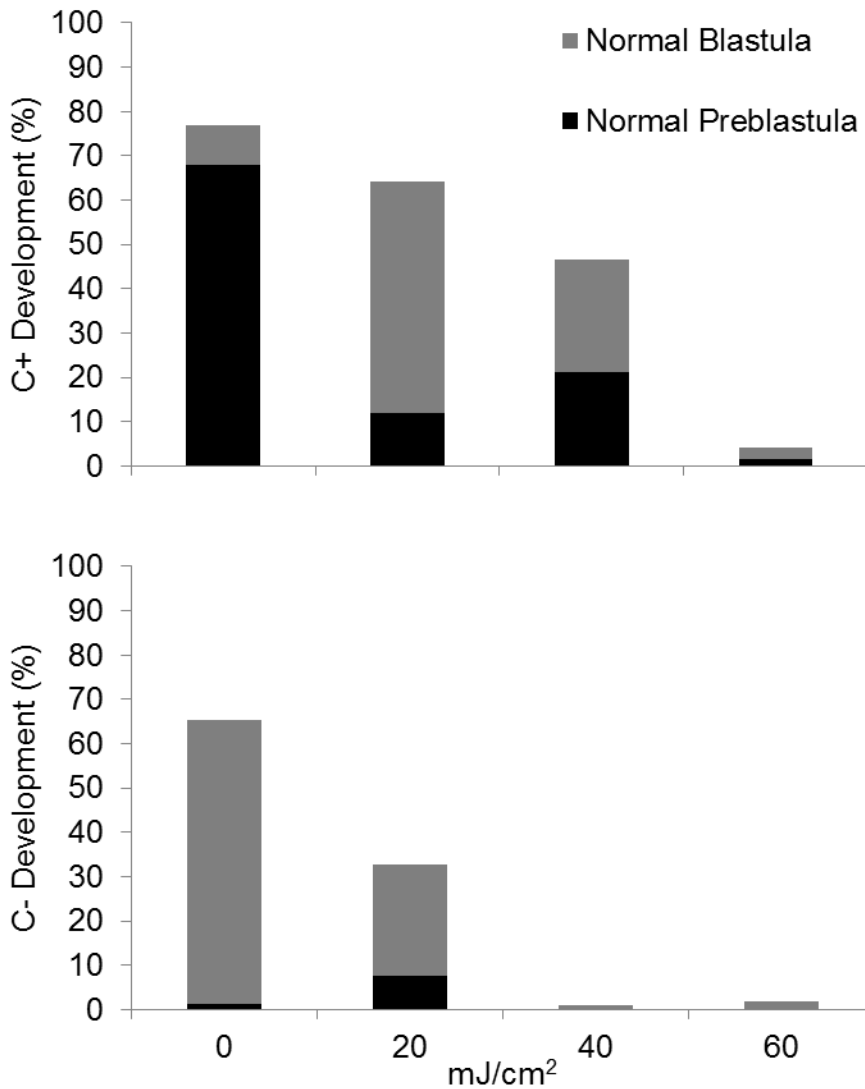


Figure 2. Percent of *Lytechinus variegatus* embryos (6.5 HPF) at each developmental stage in which the parents were fed a diet (A) with or (B) without supplemental β -carotene. Embryos were exposed to one of 3 intensities of UVB radiation at 1 HPF. Exposure to as little as 20 mJ/cm² UVB resulted in significant alterations in development within both diet treatments. Significantly higher numbers of normal blastula were recorded in C+ at UVB exposures of 20 and 40 mJ/cm² as compared to C-.

Survival at 34 HPF

Exposure to as little as 2 J/m² UVA (Fig. 3) and 40 mJ/cm² UVB (Fig. 4) resulted in a significant decrease in survival within both diet treatments. For embryos that received no UVR, there was no significant difference in survival between diet treatments ($p=0.086$). Significantly lower survival was found in C+ at UVB exposures of 60, 80 and 100 mJ/cm² ($p=0.0439, <0.001, <0.001$, and

<0.001, respectively)(Fig. 4). Significantly lower levels of survival were recorded in C+ at UVA levels of 2 and 4 J/m² (p=0.0341, 0.0001, respectively) (Fig. 3).

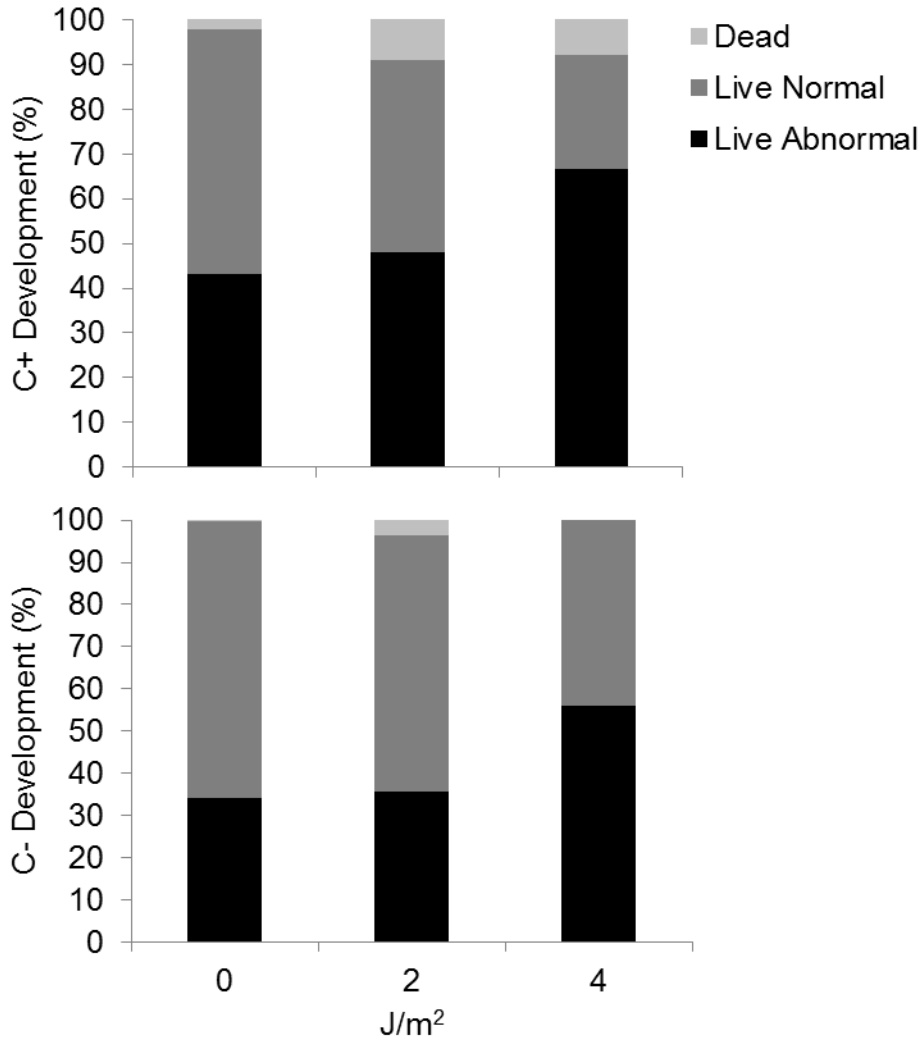


Figure 3. Percent of *Lytechinus variegatus* embryos (34 HPF) at each developmental stage in which the parents were fed a diet (A) with or (B) without supplemental β-carotene. Embryos were exposed to 2 or 4 J/m² UVA radiation at 1 HPF. Exposure to as little as 2 J/m² UVA resulted in a significant decrease in survival within both diet treatments. Exposure to as little as 2 J/m² in C+ and 4 J/m² in C- resulted in a significant decrease in the numbers of normally-developing embryos. Significantly lower survival was recorded in C+ at UVA levels of 2 and 4 J/m². Between treatments, higher percentages of normally-developing embryos were observed in C-.

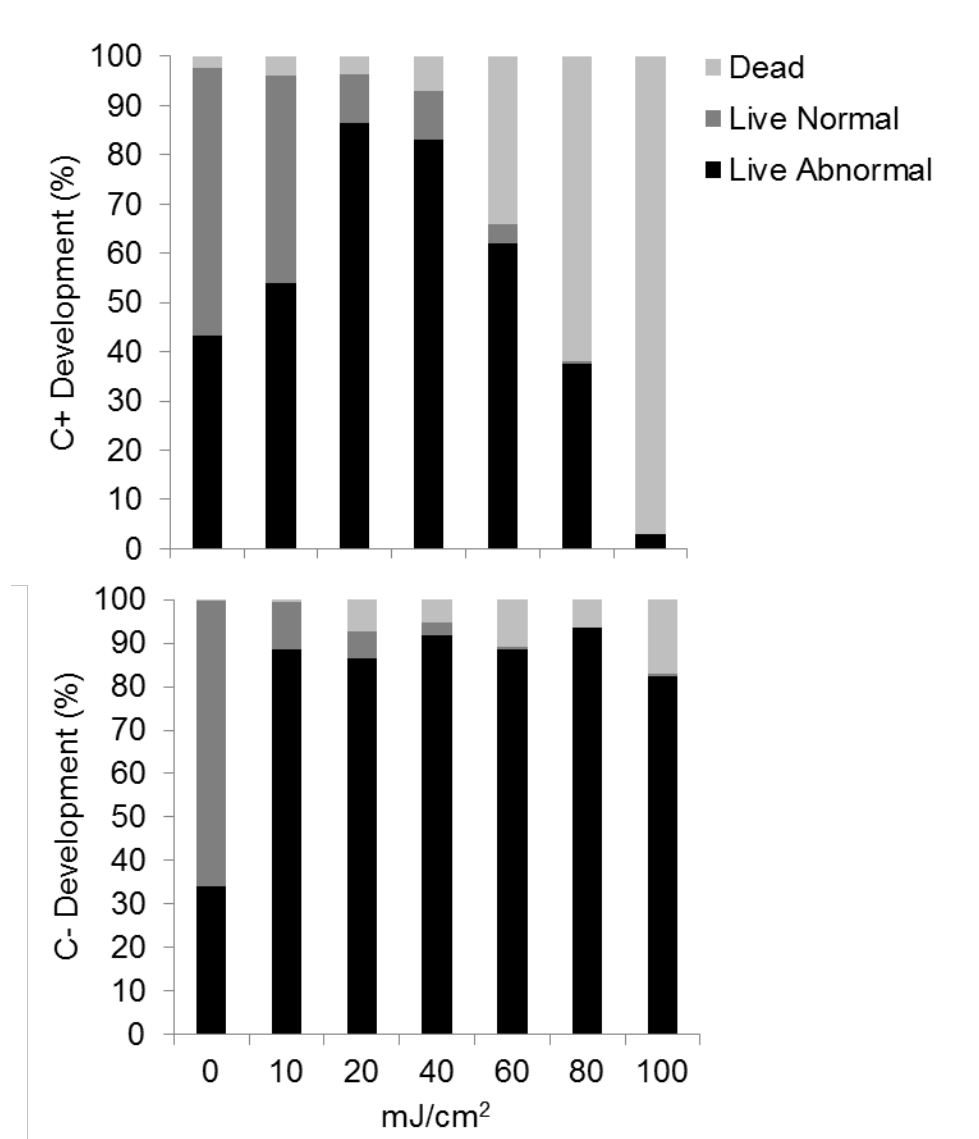


Figure 4. Percent of *Lytechinus variegatus* embryos (34 HPF) at each developmental stage in which the parents were fed a diet (A) with or (B) without supplemental β -carotene. Embryos were exposed to one of six intensities of UVB radiation at 1 HPF. Exposure to as little as 40 mJ/cm² UVB resulted in a significant decrease in survival within both diet treatments. Significantly lower survival was found in C+ at UVB exposures of 10, 60, 80 and 100 mJ/cm².

Development at 34 HPF

Exposure to as little as 2 J/m² in C+ (Fig. 3) and 4 J/m² in C- (Fig. 4) resulted in a significant decrease in developmental rate in normally-developing embryos. In treatments that received no UVR or in UVA exposures of 2 or 4 J/m², a significantly higher number of normally developed living embryos were recorded in C- as compared to C+ (p=0.007, <0.001, <0.001, respectively) (Fig. 3). Higher numbers of normally developed embryos were recorded at UVB levels of 10, 40, and 60 mJ/cm² in C+ as compared to C- (p= <0.001, 0.011, 0.043, respectively) (Fig. 4).

Survival at 55 HPF

At 55 HPF, survival was significantly decreased within C+ at all levels of UVA exposure (Fig. 5). There was no significant difference in survival at any UVA exposure in C- (Fig. 5). Exposure to as little as 20 mJ/cm² UVB resulted in a significant decrease in survival within both diet treatments (Fig. 6). There was no significant difference in survival between diet treatments that were not exposed to UVR ($p = 0.0291$). There was significantly lower survival within C+ at UVB exposures of 60, 80, and 100 mJ/cm² ($p = 0.0020, <0.001, 0.0418$, respectively), but not at 10, 20 and 40 mJ/cm² ($p = 0.2984, 0.8437, 0.6094$, respectively) (Fig. 6). Higher survival was recorded in C- at UVA levels of 1 and 2 J/m² ($p = <0.001, 0.0004$, respectively). There was no significant difference in survival between the diet treatments at a UVA exposure of 4 J/m² ($p = 0.0903$) (Fig. 5).

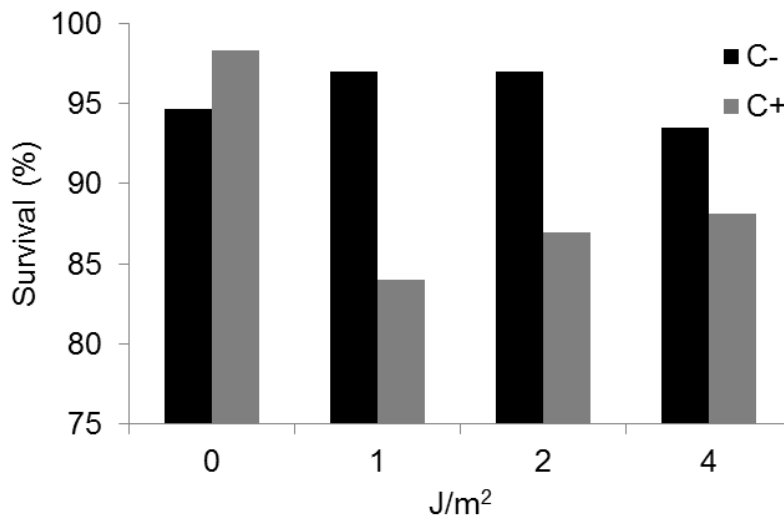


Figure 5. Percent survival at 55 HPF of *Lytechinus variegatus* embryos in which the parents were fed a diet (A) with or (B) without supplemental β -carotene. Embryos were exposed to one of 3 intensities of UVA radiation at 1 HPF. Survival was significantly decreased within C+ at all levels of UVA exposure. Between treatments, highest survival was recorded in C- at UVA levels of 1 and 2 J/m².

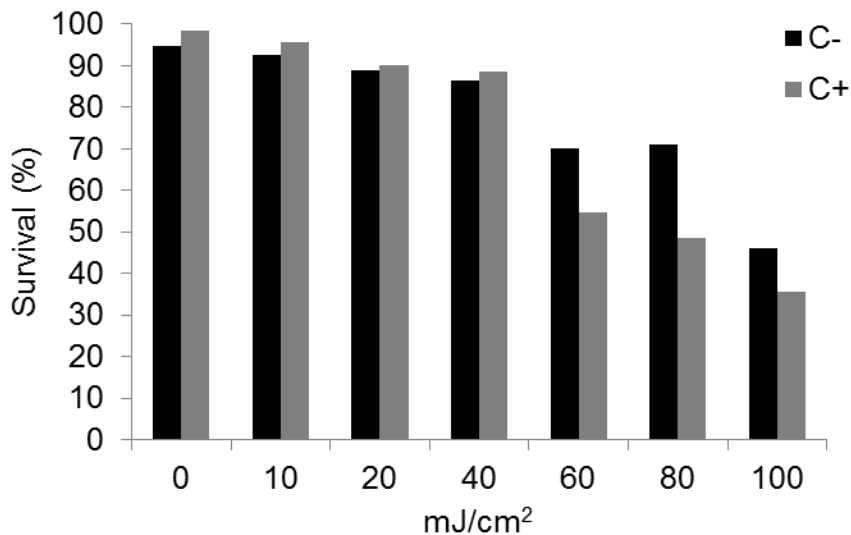


Figure 6. Percent survival at 55 HPF of *Lytechinus variegatus* embryos in which the parents were fed a diet (A) with or (B) without supplemental β -carotene. Embryos were exposed to one of six intensities of UVB radiation exposure at 1 HPF. Exposure to as little as 20 mJ/cm² UVB resulted in a significant decrease in survival in both diet treatments. There was significantly lower survival within C+ at UVB exposures of 60, 80, and 100 mJ/cm² between treatments

DISCUSSION

We originally hypothesized that *Lytechinus variegatus* embryos derived from broodstock fed a carotenoid supplement would be protected from UVR. However, in some cases these embryos were shown to be at a disadvantage within the parameters of our study. We suggest parental carotenoid dietary intake affected the stress response of offspring exposed to differing levels of UVA and UVB radiation. Survival and development of both dietary treatments were impacted by UVR exposure. Embryos from both treatments were more sensitive to UVB radiation than UVA, most likely the result of higher levels of genomic disruption associated with UVB (Afaq and Mukhtar 2006). The initial hypothesis that carotenoids or metabolites stored within offspring (derived from carotenoid-supplemented broodstock) would protect against all levels of UVR exposure was not supported.

Parental carotenoid dietary intake affects the developmental rate of *Lytechinus variegatus* offspring between 0 and 55 hours post fertilization. Embryos from broodstock that received supplemental carotenoids developed at a slower rate than embryos from broodstock that received no supplemental carotenoids. We suggest that inclusion of supplemental carotenoids in the broodstock diet was responsible for a decrease in developmental rate of offspring as well as an increase in developmental abnormalities observed at both 6.5 and 34 hours post fertilization. It is difficult to determine if either an increase or decrease in developmental rate is a positive outcome reflecting embryonic health. However, since a decreased rate of development was coupled with increased abnormalities, we hypothesize that the supplementation of carotenoids at the level provided to the broodstock may have resulted in an accumulation of carotenoids or related metabolites in the eggs, which reduced developmental rates and increased abnormal development.

If this hypothesis is correct, then excessive dietary carotenoids can affect normal development as well as development in the presence of additional stressors, including UVR. Higher levels of normally developed embryos found in some UVR exposure treatments may also be due to an adaptive response to a low-level insult wherein UVR repair stimulates increased DNA synthesis and cell division rates (Skov, 1999).

The biochemical composition of the sea urchin eggs in the current study was not known, but broodstock were all fed a diet to excess shown previously to promote good growth and health (Hammer et al. 2012, Heflin et al. 2013), thus, it is doubtful that they were nutritionally restricted. Dietary carotenoids in sea urchins; however, are necessary for gamete health and development (Tsushima et al., 1991; de Jong Westman et al., 1995a, b). Kawakami et al. (1998) found that carotenoids promote phagocytic engorgement of foreign materials within the soma and gonad of the sea urchin *Psuedocentrotus depressus*. Nutritive phagocytes in the gonad aid in gamete development and β -echinenone is the principal facilitator of phagocytic activity in *P. depressus*. Concentrations of the carotenoids β , β -carotene and fucoxanthin were also positively correlated with successful gamete development.

Carotenoid sequestration in tissues can have negative effects in some organisms. Grether et al. (2008) hypothesized that excess carotenoids in the female guppy *Poecilia reticulata* may be transferred to developing eggs as a means of pigment disposal. They found carotenoid deposition in eggs was strongly affected by dietary carotenoid levels. These authors also proposed that excess carotenoid deposition within female *P. reticulata* negatively affects crypsis. We suspect that excess carotenoid in the urchin C+ diet may have been transferred to eggs and reduced developmental rate via unknown mechanisms. Toxic levels of carotenoid metabolites have also been suggested to induce developmental abnormalities. Vitamin A (a product of β -carotene metabolism) has been suggested to cause birth defects in humans when consumed at levels approximately as low as 25,000 IU/d (Hathcock et al., 1990). Levels of carotenoids that maximize reproductive health of broodstock, fecundity, and F1 embryonic health in *L. variegatus* have not been determined.

Light spectra and diet have been suggested to alter carotenoid deposition in the sea urchin gonad. In a previous study, prolonged blue actinic light exposure increased the redness phenotype of *L. variegatus* gonads when dietary β -carotene supplements are proffered *ad libitum* (Taylor et al., 2014). We subsequently hypothesized that this increase in redness is due to increased concentrations of carotenoids within the gonad. In the current study, sea urchins from both diet treatments were sequestered beneath actinic lamps for 5 months, potentially increasing carotenoid deposition into eggs during this time period.

Levels of carotenoids in the gonads or ova were not measured in either treatment. We cannot directly link the carotenoid concentration of the eggs to survival and developmental outcomes in the offspring. We only correlate these outcomes with the diet of the parents. A significant increase in mortality due to parental β -carotene supplementation was measured at 60, 80, and 100 mJ/cm² UVB exposure. We hypothesize that these intensities of UVB radiation promoted the formation of toxic byproducts from internal stores of β -carotene, which negatively impacted survival and development. Embryos from parents that did not receive β -carotene supplements may not have had significant stores of carotenoids within their soma. Siems et al. (2005) reported that β -carotene

exerts protective antioxidant activity during moderate oxidative stress. Heavy oxidative stress, however, will cleave carotenoids into oxidative products that accumulate within the cell and damage mitochondria, DNA, lipids, and the nucleus (Siems et al., 2005). Oxidative damage to DNA increases the risk of cancer and developmental abnormalities. These data support the findings of Siems et al. (2005), as well as those of the Alpha-Tocopherol, Beta-Carotene-Cancer-Prevention study, and the Beta Carotene and Retinol Efficacy Trial (Omenn et al., 1996, The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994).

While dietary carotenoids are necessary for successful gamete development, appropriate dietary levels and types of carotenoids have not been established for sea urchins. We hypothesize that levels of β -carotene and/or other carotenoids or xanthophylls in the C+ diet supersede the nutritional requirement of *L. variegatus* and are stored in excess. Excess β -carotene within the embryo may be toxic or increase its susceptibility to oxidative or other types of stress. In this study we were not able to evaluate the levels of carotenoids in the embryos and hypothesize that levels of inclusion within the eggs were proportional to those found in the gonads of broodstock fed supplemental dietary carotenoids. Changes in color phenotype of the gonads of broodstock fed supplemented dietary carotenoids confirm that carotenoids or their metabolites are incorporated into the gonad tissues and, presumably, transferred to the eggs (Taylor et al., 2014). Further research is necessary to evaluate the requirements and function of carotenoids in sea urchin tissues.

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