Comparative Biochemistry and Physiology, Part C 151 (2010) 343–350

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

# Influence of the dark/light rhythm on the effects of UV radiation in the eyestalk of the crab *Neohelice granulata*

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ARTICLE INFO

*Article history:*
Received 6 November 2009
Received in revised form 20 December 2009
Accepted 22 December 2009
Available online 7 January 2010

*Keywords:*
Antioxidant capacity against peroxyl radicals
Catalase activity
Crustaceans
Lipid peroxidation
Oxidative stress
Photoperiod
Reactive oxygen species
Visual system

ABSTRACT

Crustaceans are interesting models to study the effects of ultraviolet (UV) radiation, and many species may be used as biomarkers for aquatic contamination of UV radiation reaching the surface of the Earth. Here, we investigated cell damage in the visual system of crabs *Neohelice granulata* that were acclimated to either 12L:12D, constant light, or constant dark, and were exposed to UVA or UVB at 12:00 h (noon). The production of reactive oxygen species (ROS), antioxidant capacity against peroxyl radicals (ACAP), lipid peroxidation (LPO) damage, catalase activity, and pigment dispersion in the eye were evaluated. No significant differences from the three groups of controls (animals acclimated to 12L:12D, or in constant light, or not exposed to UV radiation) were observed in animals acclimated to 12L:12D, however, crabs acclimated to constant light and exposed to UV radiation for 30 min showed a significant increase in ROS concentration, catalase activity, and LPO damage, but a decrease in ACAP compared with the controls. Crabs acclimated to constant darkness and exposed to UV for 30 min showed a significantly increased ROS concentration and LPO damage, but the ACAP and catalase activity did not differ from the controls (animals kept in the dark while the experimental group was being exposed to UV radiation). Pigment dispersion in the pigment cells of eyes of animals acclimated to constant light was also observed. The results indicate that UVA and UVB alter specific oxidative parameters; however, the cell damage is more evident in animals deviated from the normal dark/light rhythm.



## 1. Introduction

Ultraviolet (UV) radiation comprises electromagnetic wavelengths between 200 and 400 nm. In order to analyze its capacity to cause damage in cell targets, UV is divided into UVA (320–400 nm), UVB (290–320 nm), and UV-C (200–290 nm). The wavelengths of UV that include UVA and UVB are also called Solar UV (Diffey, 2002). The increased incidence of UV radiation on the Earth's surface is receiving more attention because it can produce biological changes and some impact on biodiversity (McKenzie et al., 2007). However, additional studies are necessary in order to understand the responses of biological systems to UV radiation damage.

The first and main target-structure for UV radiation in animals is the body surface, including the skin and eyes. The influence of UV radiation on the human skin has been investigated: in general, the effects of UV radiation on the epidermis are harmful (Tran et al., 2008), except for stimulation of vitamin D synthesis (Holick, 2008). The adverse effects of UV radiation include immunosuppression (Timares et al., 2008), production of reactive oxygen species (ROS) (Heck et al., 2003), photoaging, DNA mutation, and cancer (Albert and Ostheimer, 2003). In the eye, damage has been reported in the cornea, characterized by inflammation; in the lens, with cataracts the most common pathology (Sliney, 2001; Meyer-Rochow, 2000); and in the retina, where apoptosis, especially in the photoreceptors, was observed (Miguel et al., 2003). The mechanism underlying this damage is oxidative stress, which results from an imbalance between reactive oxygen species (ROS) and antioxidants, i.e., oxidative stress occurs when ROS production exceeds the capacity of the antioxidant system to repair the deleterious effect of ROS. The most commonly encountered ROS in biological systems include the hydroxyl radical, the superoxide anion, hydrogen peroxide ($H_2O_2$), and singlet oxygen (Sies, 1991). ROS results from the incomplete reduction of oxygen species and can be produced by both exogenous, such as solar radiation, and endogenous sources, such as the mitochondrial

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doi:10.1016/j.cbpc.2009.12.011

electron transport chain and the endoplasmic reticulum (Dean et al., 1997), the activity of enzymes, *viz.* cytochrome P450, xanthine oxidase, urate oxidase, and D-amino acid oxidase (Stadtman and Levine, 2000).

Animals may respond to environmental conditions, the most important being the photoperiod. Circadian and exogenous daily variations, including those related to locomotor and brain activity, as well as environmental temperature and light fluctuations, result in corresponding daily patterns of reactive oxygen species (ROS) (Hardeland et al., 2003). Some investigators have proposed that circadian clocks organize metabolic functions into a coherent daily schedule, assuring their synchrony with environmental changes (Wijnen and Young, 2006).

If in vertebrates, mainly mammals, the adverse effects of UV radiation are well known, this is not true for invertebrates. To our knowledge, in crustaceans only Gouveia et al. (2005) have reported oxidative and DNA damage in the cephalothorax and pereiopod epithelia in the crab *Neohelice granulata*, and Miguel et al. (2002) reported morphological damage in the retina and *lamina ganglionaris* cells in the mangrove crab *Ucides cordatus*.

The crab *N. granulata* (=*Chasmagnathus granulatus*) is a semi-terrestrial animal of the southern coast of Brazil, Uruguay, and Argentina, and it has been used by our group as a model for studies on the effects of UV. This species shows pigment dispersion in the melanophores during UV exposure, which disappears when the stimulus ceases (Gouveia et al., 2004; Vargas et al., 2008). Although the effects of UV radiation on the epithelium of this crab have been studied, the effects of UV on the visual system have not so far been investigated. Thus, the aim of this study was to investigate the effects of UV radiation on the visual system of *N. granulata* in response to oxygen species production, using a biochemical approach and the evaluation of pigment dispersion. This may help to understand how crustaceans, which are an integral part of estuarine environments and contribute substantially to their dynamics, are affected by UV rays. In addition, because UVB radiation reaching the surface of the Earth has been increasing in recent decades (Casiccia et al., 2003; Bertagnolli et al., 2007; Kirchhoff et al., 2000), these animals may eventually be used as biomarkers for aquatic contamination.

## 2. Materials and methods

### 2.1. Animals

Adult male crabs *N. granulata* weighing 7.0 ± 0.5 g (mean ± S.E.M) were collected in salt marshes near the city of Rio Grande, Brazil. They were transferred to the laboratory for an acclimation period of at least 10 days in tanks under constant conditions of temperature (20 °C) and salinity (20 ppt), and in three photoperiods, consisting of 12L:12D, constant light, and constant darkness. In the 12L:12D regime, the lights went on at 6:00 h and went off at 18:00 h. All procedures adopted in this study were performed after approval by the National Environmental Committee (IBAMA document number 1.637.714), and every effort was made to minimize animal suffering.

### 2.2. Assays

Within each set of crabs acclimated to one of the three different photoperiods, a group was exposed to UVA radiation, another group was exposed to UVB radiation, and still another group was the control. This control group was constituted by the animals that were not exposed to UV radiation (UVA or UVB), but to visible light (the animals acclimated to 12L:12D and constant light) or maintained in the dark (animals acclimated to constant darkness) during the same period of time that the experimental groups were exposed to UV radiation.

Therefore, after the acclimation period, two groups of crabs were irradiated at 12:00 h (noon), since this is the time when UV radiation is maximal, with different doses of UVA (1.575 J/cm$^2$) and UVB (1.294 J/cm$^2$), respectively, for 30 min. The UVA (VL: 115 L, 30 W) or UVB (VL: 115 C, 30 W; Vilber Lourmat, Marne Lavalee, France) lamps were monitored using a radiometer/photometer (model IL 1400A, International Light, Newburyport, MA, USA). The UVA lamp irradiation was 1.39 mW/cm$^2$ UVA, with contamination of 0.006 mW/cm$^2$ UVB and 0.000928.0 mW/cm$^2$ of visible light. The UVB lamp irradiation was 1.195 mW/cm$^2$, produced with contamination of 0.493 mW/cm$^2$ of UVA and 0.000113 mW/cm$^2$ of visible light. Both lamps showed no contamination with UV-C. The control group, formed with crabs that were not irradiated with UV (animals acclimated to 12L:12D and constant light), was maintained under fluorescent lamps (Philips TLT 40 W/75, São Paulo, Brazil) irradiating 96.0 mW/cm$^2$ visible light. After the exposure, the animals were killed by severing the supra-esophageal ganglion, and the eyes were removed for further analyses.

#### 2.2.1. ROS production

Two hundred and seventy eyestalks (90 for each group: control, exposed to UVA, and exposed to UVB) were weighed and homogenized (1:20 w/v) in a cold (4 °C) buffer solution containing sucrose (250 mM), PMSF (1 mM), and EDTA (5 mM), with pH adjusted to 7.6. The samples were centrifuged twice (2000*g*, 4 °C for 20 min) and the supernatant was collected and centrifuged again (10,000 *g*, 4 °C for 45 min). The supernatant resulting from this last centrifugation was used for the determination of the ROS (Viarengo et al., 1999). For ROS detection, we used 2′,7′-dichlorofluorescein-diacetate ($H_2$DCF-DA, Molecular Probes). This molecule in the presence of ROS generates a fluorochrome, detected at 488 and 525 nm wavelengths for excitement and emission, respectively. The analyses were carried out in a fluorescence microplate reader (Victor 2, Perkin Elmer) with readings every 5 min for 60 min. The total fluorescence production was calculated by integrating the fluorescence units (FU) during the period of the measurement, after adjusting the FU data to a second-order polynomial function. The ROS concentration was referred to the total protein content present in the biological sample and expressed in FU (mg of protein)$^{-1}$.

#### 2.2.2. Antioxidant capacity against peroxyl radical analysis

Another 270 eyestalks (separated into three groups as explained in Section 2.2.1) were weighed and homogenized (1:20 w/v) in a cold (4 °C) buffer solution containing sucrose (250 mM), PMSF (1 mM), and EDTA (5 mM), with pH adjusted to 7.6. The samples were centrifuged twice at 2000*g*, 4 °C, for 20 min, and the supernatant was collected and centrifuged again (10,000*g*, 4 °C, for 45 min). The supernatant of this last centrifugation was then employed for the analyses. The antioxidant capacity against peroxyl radicals (ACAP) was measured according to the method of Amado et al. (2009). Briefly, 10 µL of the supernatant prepared for the enzyme analysis was pipetted into a white 96-well microplate, six wells per sample. The reaction buffer (127.5 µL) containing 30 mM HEPES (pH 7.2), 200 mM KCl, and 1 mM $MgCl_2$ was added to the wells with the samples. In three of the six wells of each sample, 7.5 µL of 2,2′-azobis 2 methylpropionamidine dihydrochloride (ABAP; 4 mM; Aldrich) was added, and the same volume of ultrapure water was pipetted into the other three wells. The microplate was inserted into a fluorescence microplate reader (Victor 2, Perkin Elmer), at a programmed temperature of 35 °C, and the peroxyl radicals were produced by thermal decomposition of ABAP. Immediately before reading, 10 µL of the fluorescent probe 2′,7′-dichlorofluorescein-diacetate ($H_2$DCF-DA) was added to the wells at a final concentration of 40 µM (Ferreira-Cravo et al., 2007). $H_2$DCF-DA is cleaved by esterases present in samples, and the non-fluorescent compound $H_2$DCF is oxidized by ROS to the fluorescent compound DCF, which is detected at

wavelengths of 488 and 525 nm, for excitation and emission, respectively. The thermal decomposition of ABAP and ROS formation were monitored with readings every 5 min for 60 min. According to Regoli and Winston (1999) and Regoli (2000), non-enzymatic low-molecular-weight scavengers (GSH, ascorbic acid, uric acid, and vitamin E) generally account for 70% of the total scavenging capacity towards peroxyl radicals. Therefore, if enzymatic inhibition occurs due to the high temperature (since crabs are poikilotherms) needed for the ABAP decomposition in the peroxyl radical (35 °C), the decrease of the antioxidant capacity should be a minor problem. The total fluorescence production was calculated by integrating the fluorescence units (FU) along with the time of the measurement, after adjusting the FU data to a second-order polynomial function. The results were calculated as the difference in area of FU × min in the same sample, with and without ABAP addition, and standardized to the ROS area without ABAP (background area). The inverse of the relative difference between the ROS area with and without ABAP was considered as a measure of the antioxidant capacity: the greater the difference in area, the higher the antioxidant capacity, since high fluorescence levels were obtained after adding ABAP, signifying a low competence to neutralize peroxyl radicals. Thus, the calculation for the antioxidant competence is:

$$1 / (ROS_{area}ABAP - ROS_{area}background) / ROS_{area}background.$$

#### 2.2.3. Catalase activity

Still another 270 optic ganglia (separated into three groups as explained in Section 2.2.1) were weighed and homogenized (1:20 w/v) in a cold (4 °C) buffer solution containing Tris base (20 mM), EDTA (1 mM), dithiothreitol (1 mM, Sigma), KCl (150 mM), and PMSF (0.1 mM), with pH adjusted to 7.6. The homogenates were centrifuged at 9000 *g*, 4 °C, for 30 min, and the supernatant was then employed for the analyses. The catalase (EC 1.11.1.6) activity was analyzed according to Beutler (1975), determining the initial rate of $H_2O_2$ (50 mM) decomposition at 240 nm. The results were expressed in CAT units: one unit is the amount of enzyme that hydrolyzes 1 μmol $H_2O_2$ per min and per mg of protein, at 25 °C and pH 8.0. This procedure was performed using a digital spectrophotometer (Biomatte 3).

#### 2.2.4. Lipid peroxidation

Two hundred and seventy eyestalks (separated into three groups as explained in Section 2.2.1) were weighed and homogenized (1:20 w/v) with methanol [in a cold (4 °C) condition and centrifuged at 1000 *g*, 4 °C, for 10 min]. The supernatant was used for the analyses. The methodology, termed FOX (Hermes-Lima et al., 1995; Monserrat et al., 2003), is based on the oxidation of Fe(II) under acidic conditions, and measures the quantity of lipid peroxides. For the lipid peroxidation (LPO) measurements, $FeSO_4$ (1 mM), $H_2SO_4$ (0.25 M), xylenol orange (1 mM, Sigma) and MilliQ water were added sequentially. Samples (30 μL) or methanol (blanks) were added and incubated for 450 min. Subsequently, the absorbance (550 nm) was determined using a microplate reader (Victor 2, Perkin Elmer), and cumene hydroperoxide (CHP, Sigma) was employed as a standard. LPO was expressed as cumene hydroperoxide (CHP) equivalents per g of wet mass.

### 2.3. Histological analysis

The eyestalks of nine crabs acclimated to the three photoperiods analyzed were dissected at 12:00 h, fixed in 4% paraformaldehyde for 4 h in 0.1 M phosphate-buffered saline (PBS), and dehydrated in a graded ethanol series. The material was embedded in paraffin, and 12 μm sections, obtained using a rotatory microtome, were collected on slides. The sections were stained with hematoxylin and eosin, for observation by means of an Olympus BX50 microscope connected to a CCD camera (Pro-series, High Performance), and provided with commercial software for image acquisition using Image Pro.

### 2.4. Statistical analysis

The statistical analyses were carried out by analysis of variance (ANOVA) followed by the Newman–Keuls test ($a = 0.05$). Normality and variance homogeneity were verified as ANOVA assumptions. Mathematical transformations were performed when necessary (Zar, 1984). The results of all experiments were expressed as a percentage compared to the control group.

## 3. Results

The control groups for 12L:12D, for constant light, and for constant darkness (none of them exposed to UV) showed different values for ROS, LPO, ACAP, and catalase activity according to the acclimation regime (Table 1). The ROS concentration was low in animals acclimated to constant darkness, and did not differ from animals acclimated to 12L:12D and constant light. The ACAP test showed higher values in those animals acclimated to constant darkness and constant light than in the animals acclimated to 12L:12D. The catalase activity showed no significant difference in the different acclimation schedules employed, whereas the LPO values were low in the animals acclimated to constant light and constant darkness.

### 3.1. ROS concentration

The animals acclimated to 12L:12D and exposed to UVA or UVB showed no significant difference when compared to the control groups for each treatment (Fig. 1A). However, the ROS concentration increased in the animals acclimated to constant light (Fig. 1B) and constant darkness (Fig. 1C) when exposed to UV radiation.

### 3.2. Antioxidant capacity against peroxyl radicals

The ACAP test performed in animals acclimated to 12L:12D (Fig. 2A) and to constant darkness (Fig. 2B) after being exposed to UV radiation showed no significant difference when compared with the control group. However, when the animals were acclimated to constant light, the ACAP result was significantly lower in animals exposed to UVA and UVB than in the controls (Fig. 2C).

### 3.3. Catalase activity

In the crabs acclimated to 12L:12D, the catalase activity increased after exposure to UVB when compared with controls (Fig. 3A). Additionally, in animals acclimated to constant light, after the exposure to UVA and UVB the catalase activity was higher than in the controls (Fig. 3B). In contrast, in animals acclimated to constant

**Table 1**
Values for ROS, LPO, ACAP and catalase activity of control groups (mean ± SEM) in 12L–12D ($n = 5$) photoperiod, constant light ($n = 5$) and constant darkness ($n = 5$) at 12:00 h.

| Assay | 12L:12D | Constant light | Constant dark |
|---|---|---|---|
| ROS concentration (FU (g of protein)$^{-1}$) | $1887.6 \pm 417.3^a$ | $2176.0 \pm 89.5^a$ | $606.8 \pm 100.9^b$ |
| LPO (CHP (g of wet mass)$^{-1}$) | $130.78 \pm 8.48^a$ | $102.75 \pm 6.32^b$ | $41.11 \pm 9.35^c$ |
| ACAP | $0.0046 \pm 0.00032^a$ | $0.048 \pm 0.008^b$ | $0.059 \pm 0.009^b$ |
| Catalase activity (U CAT (mg of protein)$^{-1}$) | $24.98 \pm 2.83^a$ | $43.66 \pm 11.11^a$ | $27.04 \pm 1.81^a$ |

Different letters represent statistically significant differences for each variable ($P < 0.05$).

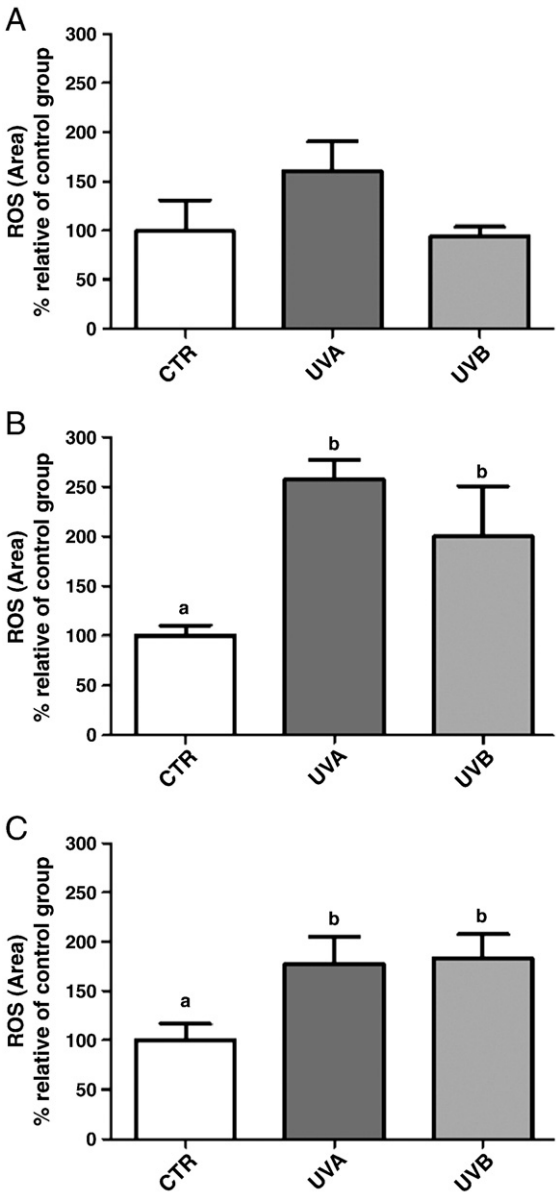


**Fig. 1.** Concentration at 12:00 h of reactive oxygen species in eyestalks of the crab *Neohelice granulata* acclimated to (A) 12L:12D, (B) constant light, (C) constant darkness, and exposed to UV radiation. Each point represents a percentage of the mean ± S.E.M ($n = 5$).

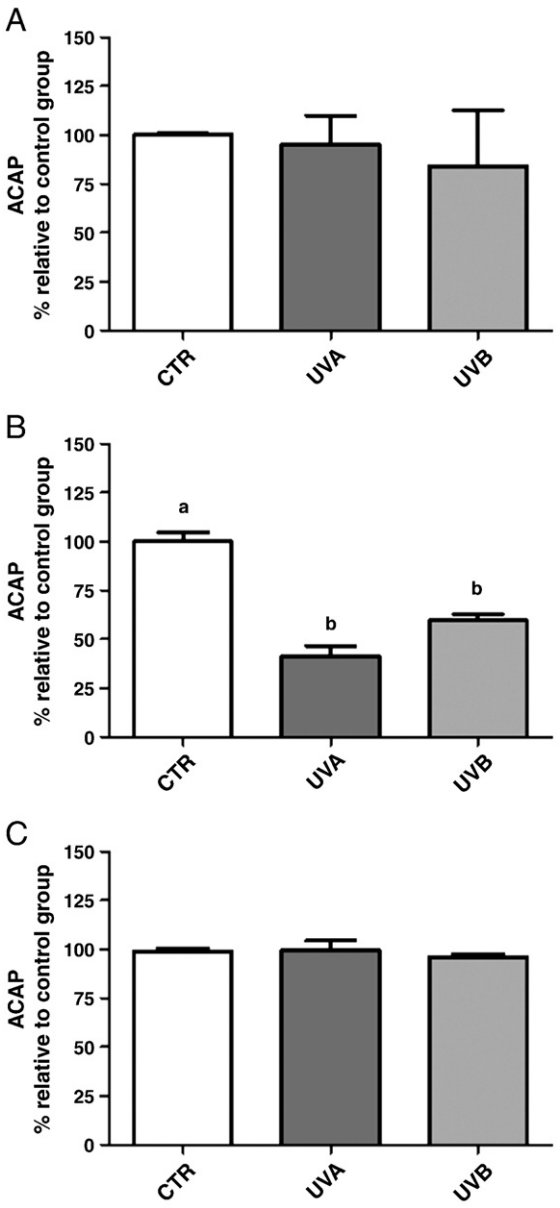


**Fig. 2.** Antioxidant competence against peroxyl radical (ACAP) at 12:00 h of eyestalks from the crab *Neohelice granulata* acclimated to (A) 12L:12D, (B) constant light, (C) constant darkness, and after exposure to UV. Each point represents a percentage of the mean ± S.E.M ($n = 5$).

darkness and exposed to UVA and UVB, catalase activity showed no significant difference from the control group (Fig. 3C).

### 3.4. Lipid peroxidation

Regarding LPO, animals acclimated to 12L:12D and exposed to UVA or UVB did not show any significant differences when compared with the control group (Fig. 4A), but when crabs acclimated to constant light and constant darkness were exposed to UV radiation, LPO values increased significantly, as shown in Fig. 4B and C, respectively.

### 3.5. Histological analysis

The visual system of decapod crustaceans consists of ommatidia composed of a cornea, a crystalline cone lens system, and a retina formed by photoreceptors and pigment glial cells. The photoreceptors are connected via the fasciculated zone to a group of three sequentially arranged optic ganglia: the *lamina ganglionaris*, the external medulla, and the internal medulla. The basement membrane limits the retina proximally (Grassé et al., 1976; Allodi et al., 1995) (Fig. 5A).

The sections of eyestalks of the crabs acclimated to 12L:12D and fixed at 12:00 h showed few pigments in the retina near the basement membrane (Fig. 5B), and the pigments were more distributed in the fasciculated zone. In the sections obtained from the retina of the crabs acclimated to constant light (Fig. 5C) and to constant darkness (Fig. 5D), pigments were clearly seen in the retina near the basement membrane and were more aggregated in the fasciculated zone (Fig. 5D). It is important to note here that in decapods maintained in normal photoperiods, light-adapted eyes show dispersed pigments, whereas during the night, most of the eye pigments are withdrawn (Fricke, 1931; Meyer-Rochow and Lindström, 1988). Therefore, the pigment position seen in eyes of crabs maintained in constant light and constant darkness indicates that these crabs are on different photoperiod schedules.

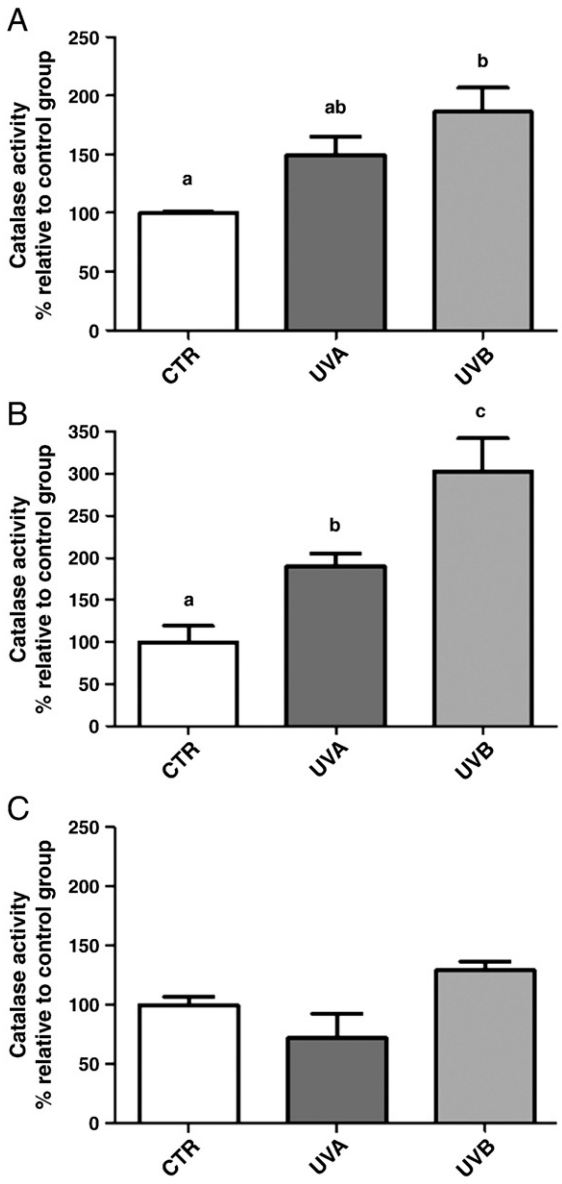


**Fig. 3.** Catalase activity determined at 12:00 h of eyestalks of the crab *Neohelice granulata* acclimated to (A) 12L:12D, (B) constant light, (C) constant darkness, and exposed to UV radiation. Each point represents a percentage of the mean ± S.E.M ($n = 5$).

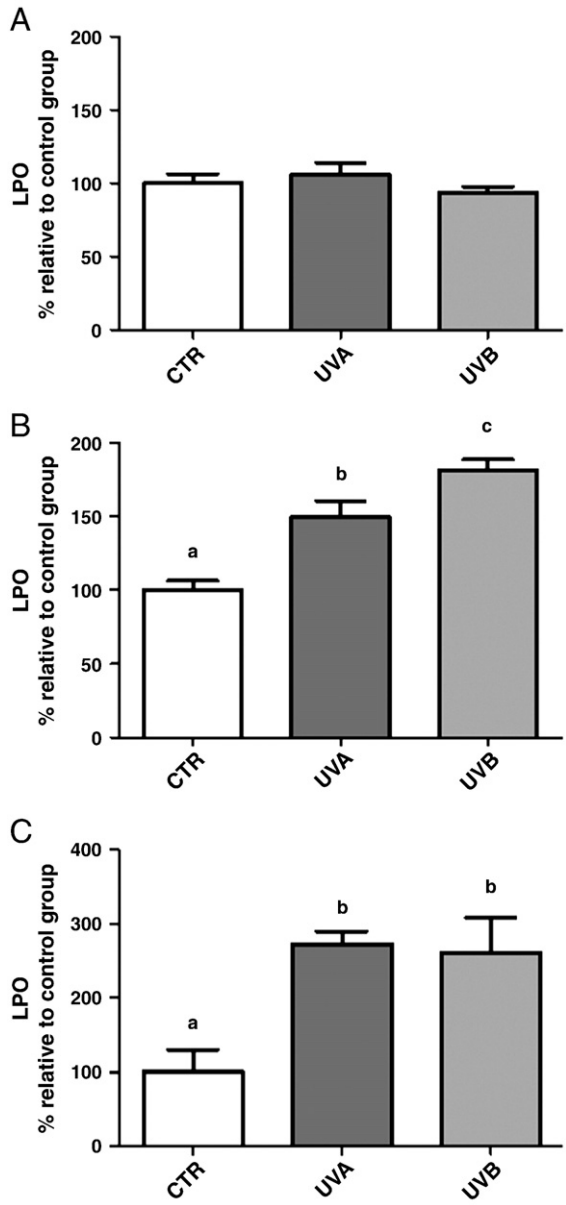


**Fig. 4.** Lipid peroxidation (LPO) of eyestalks from the crab *Neohelice granulata* acclimated to (A) 12L:12D, (B) constant light, (C) constant darkness, exposed to UV radiation and analyzed at 12:00 h. Each point represents a percentage of the mean ± S.E.M ($n = 5$).

## 4. Discussion

Photoperiod plays an important role in animal metabolic functions (Hardeland et al., 2003), and when their dark/light rhythm is disturbed, animals can be susceptible to oxidative stress (Fanjul-Moles and Prieto-Sagredo, 2003; Fanjul-Moles et al., 2009; Prieto-Sagredo et al., 2000). In this study, as summarized in Table 1, we showed that non-irradiated animals acclimated to different conditions altered their oxidative status: the ROS concentration decreased in animals acclimated to constant darkness, and the ACAP increased in animals acclimated to constant light and constant darkness. The catalase activity showed no significant difference from the controls; however, the LPO values decreased in animals acclimated to constant conditions.

The ability of UV radiation to produce noxious substances is well known. For example, the shorter, higher-energy wavelengths of UV radiation are potential initiators of photochemical reactions that induce oxidative stress through the generation of ROS (Lassen et al., 2008). The eye surface, which is generally exposed to atmospheric oxygen and sunlight, including UV radiation, generates ROS and causes oxidative stress in biological systems (Wenk et al., 2001). In the visual system of *N. granulata*, UV radiation increased the ROS concentration in the eyestalk of animals acclimated to constant light. Similarly, it has been shown that both UVA and UVB can produce ROS in lens proteins of mammals (Andley and Clark, 1989; Linetsky and Ortwerth, 1995, 1997; Linetsky et al., 1996), in the lens as a whole (Babu et al., 1995; Spector et al., 1995), and in cultured cornea epithelial cells (Shimmura et al., 1996).

The increase of the ROS concentration following UV irradiation has the capacity to damage all biomolecules, including lipids (Lassen et al., 2008). In the eyestalks of *N. granulata*, UV radiation increased LPO levels in the animals acclimated to constant light and constant darkness. Interestingly, LPO levels of rabbit corneal cells following UV irradiation were not significantly altered, even though UV was used in relatively high doses, suggesting that the cornea antioxidative

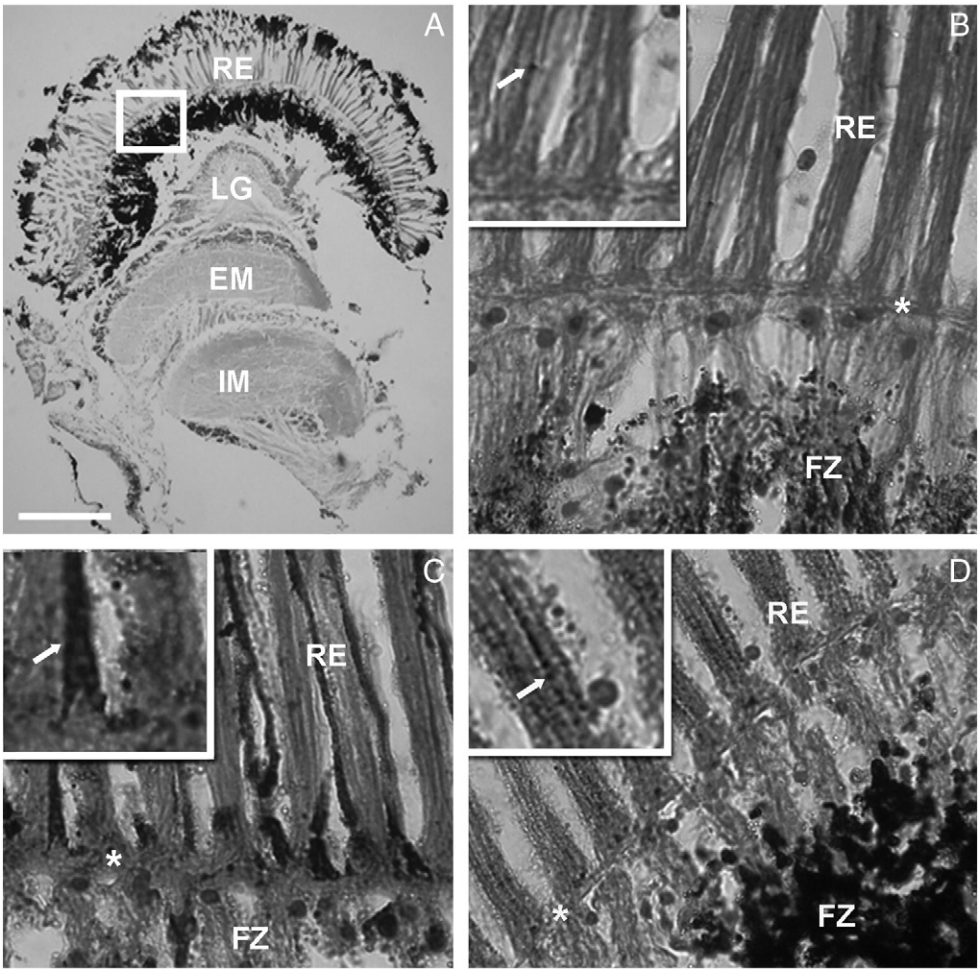


**Fig. 5.** Histological longitudinal sections of eyestalks of the crab *Neohelice granulata* stained with hematoxylin and eosin. (A) Low magnification of an eyestalk, showing the retina (RE), fasciculated zone (FZ), *lamina ganglionaris* (LG), external medulla (EM), and internal medulla (IM). The square shows the region where higher magnifications of eyestalks of animals acclimated to 12L:12D (B), to constant light (C), and to constant darkness (D) fixed at 12:00 h are shown. Asterisks represent the basement membrane. The arrows in the insets indicate the pigment migration pattern in the retina. Scale bars: A — 600 μm; B — 10 μm, insert — 7 μm; C — 10 μm; insert — 8 μm; D — 13; insert — 5 μm.

enzymes and other antioxidants, such as reduced glutathione, were able to diminish ROS generated by UV irradiation, at least under the experimental conditions used. On the other hand, UVA irradiation of lens epithelial cells resulted in a three-fold increase in lipid peroxides. The LPO in the rabbit lens appears to be associated with the increase of ROS concentration (Rogers et al., 2004). This association between ROS concentration and LPO following UV irradiation may also be occurring in the experimental procedures used in the eyestalk of *N. granulata* in this study, since it is known that LPO levels increase when there is a significant increase in the ROS concentration.

Catalase is a heme protein that reduces $H_2O_2$ to water and molecular oxygen, and is most useful to block cellular damage in tissues submitted to oxidative stress. Catalase is a constituent of the visual systems of both mammals (Atalla et al., 1987; Kenney et al., 2005; Miguel et al., 2003) and crustaceans (Miguel et al., 2005; Miguel et al., 2007). Also, it has been reported that UV radiation is able to reduce the activity of catalase in the corneal epithelial cells of rabbits (Cejkova et al., 2000). In our study, we observed different results: catalase activity increased after exposure to UVB radiation in the crabs acclimated to 12L:12D. In addition, we observed an increase in catalase activity after UVA and UVB irradiation in the animals acclimated to constant light. On the other hand, the animals acclimated to constant darkness showed no significant difference in catalase activity after exposure to UV radiation when compared to the controls. These results may be explained as Heck et al. (2003) suggested: as a consequence of the association between the levels of catalase and the quantity of UV received by the cells. In other words, accumulation of excessive ROS, generated through the action of catalase, may lead to oxidative stress. In addition, the decrease of the ACAP levels after the exposure to UV radiation of the crabs acclimated to constant light, compared to the controls, may be associated with the increase of the ROS concentration caused by UV radiation, which can induce LPO damage.

Pigments are very important to protect the cells from the damage caused by UV radiation. In mammals, tanning appears to involve cross-talk between keratinocytes and melanocytes, and results in the transfer of melanin-containing melanosomes into the more superficially located keratinocytes, where the pigment forms a “cap” over the sun-exposed surface of the nucleus (Duval et al., 2001; Yamaguchi et al., 2008). In crustaceans, Gouveia et al. (2005) reported that pigments are able to decrease damage caused by UV radiation in the cells of *N. granulata*. In this study, we observed differences in the aspect of pigment aggregation in the retina of *N. granulata*. The pigments in the eyes of the crabs acclimated to 12L:12D and fixed at 12:00 h were seen in a state which is expected for animals in their normal dark/light period, i.e., they were dispersed in the retina in order to protect the photoreceptors from light. This state differed from the animals acclimated to constant light and constant darkness, which showed more pigments near the basement membrane, and pigment aggregation in the fasciculated zone. Therefore, the changing of the photoperiod to a constant condition may have caused the loss of the protecting effect regulated by the photoperiod.

The photoperiod is a crucial environmental factor for many physiological roles of living organisms, and many molecules, such as melatonin, are mediators of the biological and physiological rhythms in animals. Interestingly, in a previous study conducted by our group (Maciel et al., 2008), we observed a biphasic profile of melatonin in crabs acclimated both to 12L:12D and to constant darkness, different from animals acclimated to constant light, which showed low levels of melatonin with no daily variation. Therefore, our results showing noxious effects of UV radiation in the eyestalk of *N. granulata* in animals acclimated to constant light could be correlated with the low levels of these molecules. In conclusion, UVA and UVB radiation can produce significant cell damage and alterations in the antioxidant defense system in the visual system of crustaceans; however, these effects are highly dependent on the photoperiod regime to which the animals are subject.

## Acknowledgments

This study was supported by CNPq, FAPERJ, and CAPES. We are grateful to Jorge Luis da Silva for technical assistance.

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