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RESEARCH ARTICLE

Effects of UV-A radiation on some antioxidant biomarkers in the freshwater zooplankter *Simocephalus vetulus* (Schoedler, 1858) (Crustacean, Cladocera)

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Abstract

This study aimed to evaluate the effects of UV-A radiation on the antioxidant biomarkers in freshwater cladoceran, *Simocephalus vetulus*. The collected cladocerans were adapted for the lab conditions and feed daily with yeast and algae. Specimens of the investigated species were classified into four experimental groups (200 animals per each). The first group was the control (unirradiated group) and the other three groups were treated (irradiated by UV-A). The three treated groups were subjected to irradiation for a period of 15, 30, and 60 minutes; respectively. After 3 days of irradiation, the activity of antioxidant enzymes (catalase CAT and superoxide dismutase SOD), total protein, lipid peroxidation marker, glutathione and nitric oxide were determined for each experimental group. Results showed significant differences in these antioxidant biomarkers between the control and treated groups. Also results showed the decrease in the antioxidant biomarkers such as LPO, SOD activity, NO, and GSH contents after 30 min of exposure.

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INTRODUCTION

The sun is our principal source of electromagnetic radiation. Ultraviolet radiation (UV) is among the many wavelengths of solar radiation. UV is subdivided into three wavelength bands: UV-A (320–400 nm), UV-B (290–320 nm) and UV-C (200–290 nm) (CIE, Commission Internationale de l'Eclairage, 2000; Leech and Williamson, 2000; Meyer-Rochow, 2000 ; Norval *et al.*, 2007). Only UV-A and UV-B reach the earth's surface and UV exposures induce biological responses (Diffey, 2002). UV-A radiation (320–400 nm) is scattered rapidly in water with biologically useful amounts to at least 100 m deep in clear aquatic environments (Losey and Hydes, 1998). Ultraviolet radiation (UV) has recently received a lot of attention since it may have negative effects on many aquatic taxa (Bancroft et al., 2007).

Cladocerans play an important role in the freshwater environment. Often they predominate in the zooplankton and littoral microfauna and are therefore of major importance in the food chain, converting phytoplankton and bacteria into animal protein and making it available to higher animals such as fish. *Simocephalus vetulus* is a littoral or shallow-water cladoceran and is relatively large and easy to handle compared to many other cladocerans (Willis *et al.*, 1995)**.**

Recently it was known that, the role of UVR doesn't depend on causing oxidative stress for crustaceans only, but also characterized as one of the most important pro-apoptotic stimuli for them (Menze *et al.*, 2010). High intensities of ultraviolet radiation are known to be harmful to aquatic biota, especially for species living in shallow, clear water bodies. Zooplankton species from such habitats are good model organisms to study the effect of changes in UV radiation, and how animals deal with this (Rautio and Korhola, 2002).

Effects of solar UV radiation on the metabolic activities (Ha¨der *et al.*, 2011; Hansson and Hylander, 2009; Ma *et al.*, 2012; Ma *et al.*, 2013) and behavioral response of zooplankton (Hansson *et al.*, 2007; Ma *et al.*, 2010; Rhode *et al.*, 2001; Rocco *et al.*, 2001; Wold and Norrbin, 2004) are well documented previously. UV

radiation suppresses the metabolic activities of zooplankton (Ma et al., 2010; Yu *et al.*, 2009), and destroys both nauplii and adults (Dattilo *et al.*, 2005; Kouwenberg *et al.*, 1999; Ma et al., 2012), or indirectly decreases their survival and fecundity by altering the nutritional value of their food (De Lange and Van Reeuwijk, 2003; Scott *et al.*, 1999).

Zooplankton have developed a variety of protective strategies against solar UV radiation during their long term evolution, such as behavioral avoidance (Hansson et al., 2007; Hylander *et al.*, 2009; Rhode et al., 2001; Rocco et al., 2001; Wold and Norrbin, 2004), effective photo-enzymatic repair systems (Ha¨der *et al.*, 2011; Hansson and Hylander, 2009; MacFadyen *et al.*, 2004), and the accumulation of photo-protective substances (Hairston, 1976; Hansson, 2000; Moeller *et al.*, 2005; Sommaruga and Garcia-Pichel, 1999; Tartarotti *et al.*, 2004).

Antioxidants are linked to resistance against UV radiation in many animals, but little is known about antioxidant protection in freshwater zooplankton. The present study can be considered as a baseline study of the major antioxidant biomarkers in the freshwater cladoceran, *Simocephalus vetulus* as large and easy handling module of freshwater zooplankton, assuming that the different levels of ambient UV-A exposure would be reflected in different levels of antioxidant biomarkers.

2. Materials and Methods

2.1. Animal and experimental design

Experiments were conducted using adult cladoceran, *Simocephalus vetulus* collected from the River Nile at Assiut city, Egypt by several horizontal tows of zooplankton net. The samples were transferred to the laboratory and cultured in normal laboratory conditions under a dark/light cycle of 12/12 h in 30 L rectangular tank containing tap water and Nile water (1:1) for one month and fed daily with algae and yeast.

The large adult cladoceran, *Simocephalus vetulus* were filtered through nylon filter from the culture. The isolated adults were kept under a dark/light cycle of 12/12 h in 1L glass beaker containing 800 ml of filtered water from the culture. *Simocephalus vetulus* were classified into four experimental groups (200 animal per each); one control (placed in normal laboratory conditions) and 3 UV-A treated. The three treated groups (15, 30 or 60 min/day) exposed to UV-A radiation for 3-day sequential. Three replicates were used for each experimental group.

2.2. UV-A source

Simocephalus vetulus were exposed to UV-A (ULTRA-VIOLET Products, Upland, CA, USA, model UVL-56) using a 6-W self-ballasted long-wave lamp (365 nm) with input voltage 220 V, 60 HZ. The UV-A source was fitted at about 20 cm above the glass beaker.

2.3. Sample preparation and biochemical analysis

After the irradiation, 1 gm of *Simocephalus vetulus* from each experiment group were homogenized in 1 ml of ice-cold 50 mM K-phosphate buffer pH 7.4, centrifuged at (8000 *g* for 20 min at 4 ◦C) and supernatant of homogenates were used directly for the evaluation of biochemical parameters. Protein concentration was determined by Folin phenol reagent method (Lowry *et al.*, 1951) and expressed as mg/ml. The activity of catalase (CAT) was determined basing on its ability to decompose H_2O_2 to H_2O and O_2 according to (Beers and Sizer, 1952). Lipid peroxidation was indirectly measured according to the method of (Ohkawa *et al.*, 1979) (thiobarbituricacid reactive substance, TBARS, test). The activity of superoxide dismutase (SOD) in tissue cytosols was determined according to its ability to inhibit the autoxidation of epinephrine at alkaline medium according to the method of (Misra and Fridovich, 1972). Nitric oxide (NO) was measured as nitrite concentration in tissue using the method of (Ding *et al.*, 1988). Glutathione (GSH) was determined using the method of (Beutler *et al.*, 1963).

2.4. Statistical analysis.

Basic statistics of the biochemical parameters of the control and exposed groups were reported as mean ± standard error (SE). The data were analyzed by one-way ANOVA followed by a post-hoc Duncan-test at 0.05 level of significance. SPSS (Statistical Package for the Social Science) package (version 16) and prism were used.

Results

The present study showed high mortality rates in *Simocephalus vetulus* after 3 days of exposure to UV-A radiation in all treated groups. The maximum percentage of dead individuals was 55% after 3 h of exposure to UV-A radiation.

The investigation of the oxidative stress biomarkers in *simocephalus vetulus* of control and treated groups exposed to different doses of UV-A radiation showed a highly significant impacts (<0.001) on the concentration of the total protein in the treated groups compared to the control group. Exposure of *Simocephalus vetulus* to UV-A radiation induced highly significant decrease in the concentration of the total protein after 15 min

and 60 min of irradiation compared to the control group (6.2 ± 0.12) mg/ml. However an increase in the concentration of the total protein was noticed after 30 min of irradiation (7.27 ± 0.29) mg/ml compared to the control group (Fig. 1. A).

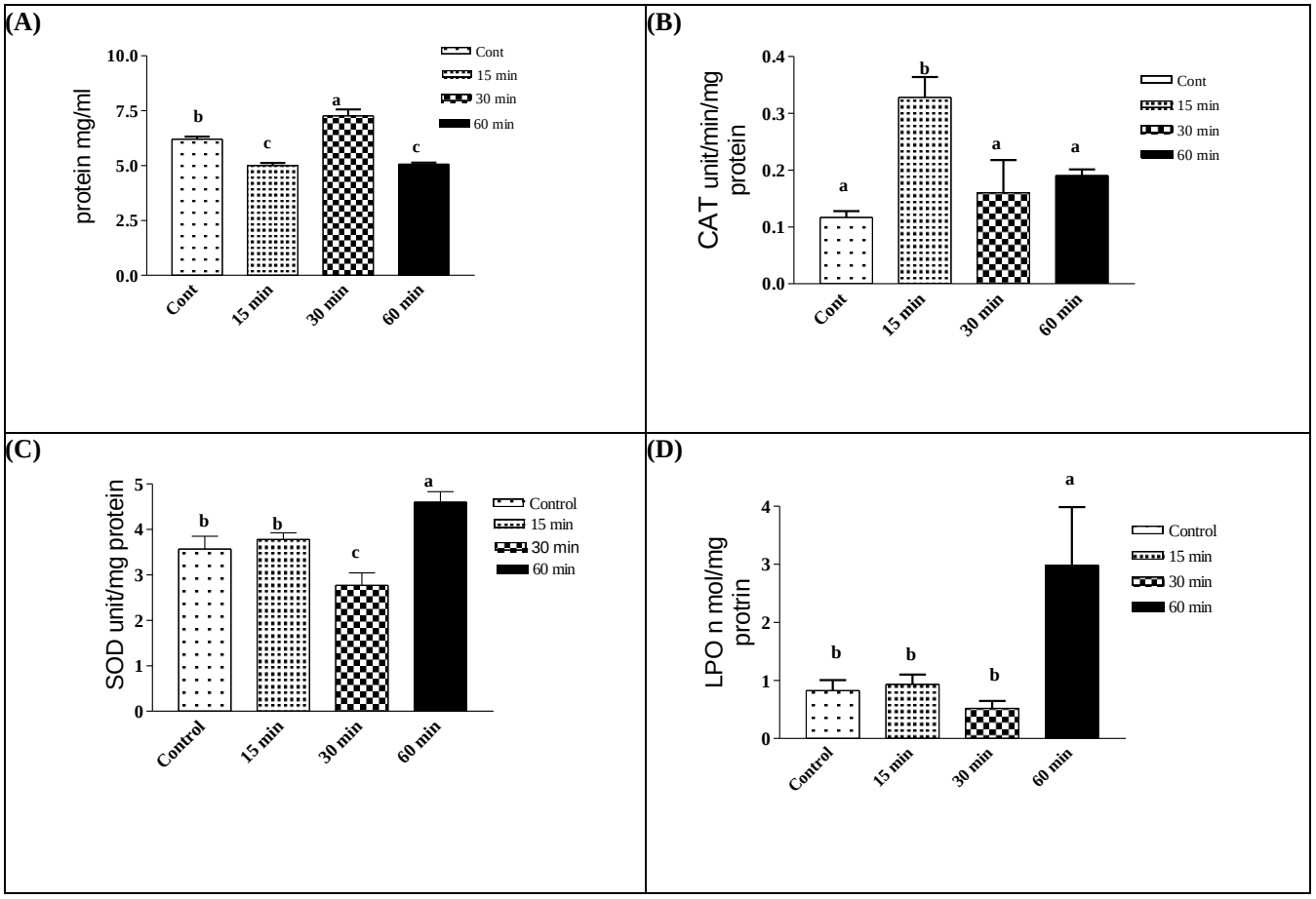
The mean value of Catalase activity (CAT) in the tissue of untreated *Simocephalus vetulus* was (0.12 ± 0.01) unit/min/mg protein, significant increase (< 0.01) in catalase activity were revealed in the irradiated groups. Decrease in Catalase activity was recorded after 30 min of irradiation (0.16 ± 0.05) unit/min/mg protein compared to 15 min and 60 min of irradiation (Fig. 1. B).

SOD activity showed significant increase after 60 min of UVA radiation (4.56 ± 0.26) unit/mg protein compared to the control group (3.5 ± 0.35) unit/mg protein. While no significant difference was recorded after 15 min of irradiation compared to the control one. Minimum significant decrease of SOD activity was observed after 30 min of exposure compared to the other different groups (Fig. 1. C).

Oxidative injury in terms of lipid peroxidation (LPO) showed no significant difference between the control group and the irradiated groups for 15 and 30 minutes. The group of irradiated *S. vetulus* for 60 minutes showed significant increase (3.09 ± 0.98) compared to the other groups (Fig. 1. D).

Nitric oxide (NO) showed highly significant differences ($p<0.001$) between the irradiated groups compared to the control one. However, it was significantly increased after 15 min of exposure compared to all other groups. Nitric oxide values showed significant decrease after 30 min and 60 min of exposure in comparing to the other groups (Fig. 1. E).

Glutathione (GSH) levels showed significant decrease ($p<0.05$) after 15 min and 30 min of irradiation compared to control group. However there is no significant difference was observed after 60 min of irradiation compared to the control group (Fig. 1. F).



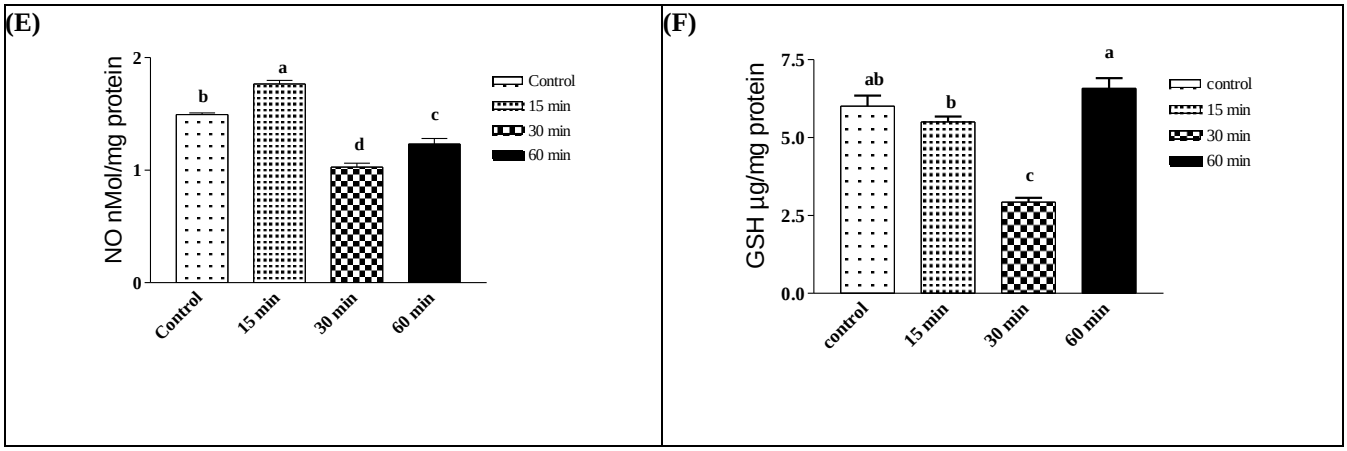


Fig.1. Concentration of antioxidant biomarkers in *Simocephalus vetulus* after exposure to UVA radiation for 15, 30 and 60 minutes. (A) Total protein (B) Catalase activity (C) Superoxide dismutase (D) Lipid peroxidation (E) Nitric oxide (F) Glutathione.

Values are the mean of three replicates (n=3) ± SE. The significant difference between the groups were analysed by one-way analysis of variance. Mean values of different subscript letters (a,b,c,d) were significantly different ($p<0.001$).

Discussion

Protein content is a reflection of the entire physiological state of the organism and a measure of its energy budget, which can be affected by environmental contaminants (De Coen and Janssen, 2003; Jemec *et al.*, 2007; Koojiman, 2000)**.** The result of this study indicated that exposure of *Simocephalus vetulus* to UV-A radiation induces highly significant decrease in the concentration of the total protein after 15 min and 60 min of irradiation; however an increase was noticed after 30 min of irradiation. The decrease in protein content after the exposure to UVA may be attributed to a physiological adaptability to compensate the UVA stress. To overcome the stress, the animal requires more energy and this energy demand may lead to protein catabolism. This result agrees with (Sancho *et al.*, 2009; Villarroel *et al.*, 2009), who observed a decrease in the protein content of *Daphnia magna* after exposure to fungicide (tebuconazole) and tetradifon, respectively. Decrease in total protein levels in freshwater clam, *Corbicula fluminea* exposed to metal toxicity was showed by (Baudrimont *et al.*, 1997). They stated that the decrease in protein content may be due to reduction in general metabolic activity generated by metal toxicity. In the present work the increase in the total protein content after 30 min of irradiation may be a result to modulate the effect of stress.

Catalase (CAT) is a well-known anti-oxidative enzyme and has been implicated in protection against H_2O_2. The present study showed significant increase in activities of CAT enzyme in *Simocephalus vetulus*, this increase may be due to UV-A radiation increases ROS production resulting in oxidative stress. Similar to the results of the present study, (Vega and Pizarro, 2000) reported an important increase in the CAT activity in *Daphnia longispina* after exposure to both UVA and UVB radiation. Also (Vlahogianni *et al.*, 2007) found an increase in CAT activity in mussels, *Mytilus galloprovincialis* exposed to water samples of coastal areas from the Saronikos Gulf of Greece polluted by heavy metals. They concluded that the increment in CAT activity may be due to a defense mechanism of the cells in order to offset the oxidative stress induced by increased H_2O_2. (Rajkumar *et al.*, 2011) stated that the increase in catalase activity is the evidence of amount of ROS neutralized. Increased activities of CAT have been reported in several invertebrate species in response to pollution (Stephensen *et al.*, 2000). CAT is considered as an important and sensitive biomarker of oxidative stress, better than SOD, revealing biological effects on the redox status of the marine organisms (Regoli *et al.*, 2002a; Regoli *et al.*, 2002b).

SOD catalytically scavenges superoxide radical which appears to be an important agent of toxicity of oxygen and provides a defense against this aspect of oxygen toxicity (Kadar *et al.*, 2005). In this work the activity of SOD showed significant increase after 60 min of irradiation, while significant decrease has occurred after 30 min of irradiation. The significant increase in SOD activity in *S. vetulus* may indicate that more protein is required to protect cells against superoxide radicals. (Dimitrova *et al.*, 1994) suggested that the excess production of superoxide radicals by themselves or after their transformation to H_2O_2 causes the oxidation of the cysteine in SOD that deactivates it. Doi *et al.* (2002) and Ozmen *et al.* (2004) suggested the depression in SOD activity may

result in cellular injury by superoxide radical. Decrease in SOD activity has been reported in *Daphnia magna* exposed to pharmaceutical compound (acetylsalicylic acid) (Gómez-Oliván *et al.*, 2014).

Lipid peroxidation (LPO) occurs by the reaction of lipid radicals and oxygen to form peroxyl radicals (Powell, 2000). In the present study, TBARS content in the *Simocephalus vetulus* tissue was significantly increased after 60 min of exposure this indicate the generation of lipid peroxide radical and oxidative stress. Similar result was obtained by (Vega and Pizarro, 2000), who observed an increase in LPO and CAT activity after exposure to UV-A radiation in *D. longispina* may be due to the presence of an inducible antioxidant protection mechanism against photo-oxidative stress. Consistent with this result, enhancement of LPO occurred in marine planktonic diatom *Ditylum brightwellii* after exposure to UV-A radiation (Rijstenbil, 2000). Lipid peroxyl radicals can damage the cells by changing the fluidity and permeability of the membrane or attacking directly DNA and other intracellular molecules, such as proteins (Mattie and Freedman, 2001).

Nitric oxide (NO) can act as a free radical scavenger and inactivate O_2^-, to prevent cell toxicity (Cooke and Tsao, 1993). Significant increase of NO after 15 min of irradiation may be due to the activation of (NOS) system. An *et al.* (2005) ; Qu *et al.* (2006) ; Seo *et al.* (2002) and Xue *et al.* (2007) reported that NO increases as a consequence of the UV-B radiation in bacteria, animal and plants. In human skin, UVA radiation induced NO production due to photo-decomposition of nitro-reactive species (NORS) and nitrite, which representing the primary basis for NO formation (Paunel *et al.*, 2005). Increase in NO human keratinocytes in skin occurred due to Increase in the activity of the enzyme NO (NOS) synthesis after exposure to UVA and UVB (Romero-Graillet *et al.*, 1996).

Glutathione is a tri-peptide non enzymatic anti-oxidant with a single cysteine residue and constitutes an important pathway of the antioxidant and detoxification defenses (Decaprio, 1997; Doyotte *et al.*, 1997; Halliwell and Gutteridge, 2007; Vlahogianni et al., 2007). In this study UV-A induced significant decrease in GSH content in *Simocephalus vetulus* after first periods of exposure compared to the control group, while no significant increase was observed after 60 min of exposure till reached the same value of the control one. The decrease in GSH content may be due to the loss of compensatory responses and conversion of GSH to its oxidized forms. This result is agreed with (H. and Lin, 1977), who suggested that GSH levels are suppressed under severe oxidative stress due to the loss of compensatory responses and oxidative conversion of GSH to its oxidized form. (Zhang *et al.*, 2004) suggested that a severe oxidative stress may suppress GSH levels due to the impairment of adaptive mechanisms. Yildirim *et al.* (2011) concluded that decreased GSH content during exposure to pollution may be due to an increased utilization of GSH, which can be converted into oxidized glutathione, and inefficient GSH regeneration. The increasing of Glutathione contents (GSH) after 60 min of irradiation may be due its role in preventing oxidative stress-associated with lipid peroxidation which subsequently makes maintaining an optimal balance of glutathione necessary for it to effectively quench peroxidation of the lipid membrane. Kerksick and Willoughby (2005) reported that, Glutathione is widely used to minimize the lipid peroxidation of cellular membranes and other such targets that is known to occur with oxidative stress.

In conclusion, UV-A seemed to be an effective stressor on the cladoceran *Simocephalus vetulus*. Significant Increase in CAT activity after the first exposure time to UV-A radiation is a defense mechanism of the cells in order to offset the oxidative stress. Decrease in total protein content after exposure occurred due to overcome the stress. Increase in LPO, SOD activity and GSH content in *Simocephalus vetulus* after long period of irradiation means oxidative damage occurred by UV-A and this damage was more effective with increasing the period of exposure.

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References

An, L.; Liu, Y.; Zhang, M.; Chen, T.; Wang, X., (2005): Effects of nitric oxide on growth of maize seedling leaves in the presence or absence of ultraviolet-B radiation. *Journal of Plant Physiology* **162**, 317–326.

Bancroft, B. A.; Baker, N. J.; Blaustein, A. R., (2007): Effects of UVB radiation on marine and freshwater organisms: a synthesis through meta-analysis. *Ecol. Lett.* **10** 332–345.

Baudrimont, M.; Lemaire-Gony, S.; Ribeyre, F.; Metivaud, J.; Boudou, A., (1997): Seasonal variations of Metallothionein concentrations in the Asiatic clam (*Corbicula fluminea*). *Comp. Biochem. Physiol. Part C: Pharmacol. Toxicol.* **118**, 361-367.

Beers, R. F.; Sizer, I. W., (1952): A spectrophotometric methods for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem* **195**, 133–140.

Beutler, E.; Duron, O.; Kelly, B. M., (1963): Improved method for the determination of blood glutathione. *J. Lab. Clin. Meth* **61**, 882–888.

CIE, (Commission Internationale de l'Eclairage, 2000): Action spectrum for photocarcinogenesis (non-melanoma skin cancers), pp. 6-32.

Cooke, J. P.; Tsao, P. S., (1993): Cytoprotective effects of nitric oxide. *Circulation* **88**, 2451–2454.

Dattilo, A. M.; Bracchini, L.; Carlini, L.; Loiselle, S.; Rossi, C., (2005): Estimate of the effects of ultraviolet radiation on the mortality of Artemia franciscana in naupliar and adult stages. *International Journal of Biometeorology* **49**, 388-395.

De Coen, W. D.; Janssen, C. R., (2003): The missing biomarker link: relationship between effects on the cellular energy allocation biomarker of toxicant stressed Daphnia magna and corresponding population characteristics. *Environ Toxicol. Chem.* **22**, 1632–1641.

De Lange, H. J.; Van Reeuwijk, P. L., (2003): Negative effects of UVB-irradiated phytoplankton on life history traits and fitness of Daphnia magna. *Freshwater Biol.* **48**, 678–686.

Decaprio, A. P., (1997): Biomarkers: Coming of age for environmental health and risk assessment. *Environ. Sci. Technol.* **31**, 1837–1848.

Diffey, B. L., (2002): What is the light? Photodermatol. *Photoimmunol. Photomed.* **18**, 68–74.

Dimitrova, M. S. T.; Tsnova, V.; Velcheva, V., (1994): Combined effect of zinc and lead on the hepatic superoxide dismutase-catalase system in carp (*Cyprinus carpio*). *Comp Biochem Physiol.* **108C**, 43–46.

Ding, A. H.; Nathan, C. F.; Stuchr, D. J., (1988): Release of reactive nitrogen intermediate and reactive oxygen intermediate from mouse peritoneal macrophages. Comparison of activating cytokine and evidence for independent production. *J. Immunol.* **141**, 2407–2412.

Doi, H.; Iwasaki, H.; Masubuchi, Y.; Nishigaki, R.; Horie, T., (2002): Chemiluminescence associated with the oxidative metabolism of salicylic acid in rat liver microsomes. *Chem. Biol. Interact.* **140**, 109–119.

Doyotte, A.; Cossu, C.; Jacquin, M.; Babut, M.; Vaseural, P., (1997): Antioxidant enzymes, glutathione and lipid peroxidation as relevant biomarkers of experimental or field exposure in the gills and the digestive gland of the freshwater bivalve *Unio tumidus*. *Aquat. Toxicol*. **39**, 93-l10.

Gómez-Oliván, L. M.; Galar-Martínez, M.; Islas-Flores, H.; García-Medina, S.; SanJuan-Reyes, N., (2014): DNA damage and oxidative stress induced by acetylsalicylic acid in Daphnia magna. *Comparative Bioch. and Physio., Part C* **164**, 21–26.

H., C. L.; Lin, S. M., (1977): Modulation of acetaminophen-induced alteration of antioxidant defense enzymes by antioxidants or glutathione precursors in cultured hepatocytes. *Biochem. Arch.* **13**, 113–125.

Ha¨der, D.-P.; Helbling, E. W.; Williamson, C. E.; Worrest, R. C., (2011): Effects of UV radiation on aquatic ecosystems and interaction with climate change. *Photoch. & Photobio. Sci.***10**, 242–260.

Hairston, N. G., (1976): Photoprotection by carotenoid pigments in the copepod Diaptomus nevadensis. *Proceedings of the National Academy of Sciences* **73**, 971–974.

Halliwell, B.; Gutteridge, J. M. C., (2007): Free Radicals in Biology and Medicine, Clarendon Press, Oxford.

Hansson, L.-A., (2000): Induced pigmentation in zooplankton: a trade-off between threats from predation and UV-damage. Proceedings of the Royal Society of London. Series B:. *Biological Sciences* **267**, 2327–2331.

Hansson, L.-A.; Hylander, S.; Sommaruga, R., (2007): Escape from UV threats in zooplankton: a cocktail of behavior and protective pigmentation. *Ecology* **88**, 1932–1939.

Hansson, L. A.; Hylander, S., (2009): Effects of ultraviolet radiation on pigmentation, photoenzymatic repair, behavior, and community ecology of zooplankton. *Photoch. & Photobio. Sci.* **8**, 1266–1275.

Hylander, S.; Larsson, N.; Hansson, L. A., (2009): Zooplankton vertical migration and plasticity of pigmentation arising from simultaneous UV and predation theats. *Limnol. and Oceang.* **54**, 483–491.

Jemec, A.; Drobne, D.; Tisler, T.; Trebse, P.; Ros, M.; Sepcic, K., (2007): Comparative toxicity of imidacloprid, of its commercial liquid formulation and of diazinon to a non-target arthropod, the micro crustacean Daphnia magna. *Chemosphere* **68**, 1408–1418.

Kadar, E.; Costa, V.; Santos, R. S., (2005): Distribution of micro-essential (Fe, Cu, Zn) and toxic (Hg) metals in tissues of two nutritionally distinct hydrothermal shrimps. *Sci Total Environ.* **358**, 143–150.

Kerksick, C.; Willoughby, D., (2005): The antioxidant role of Glutathione and N-Acetyl- Cysteine supplements and exercise-induced oxidative Stress *Journal of the International Society of Sports Nutrition.* **2(2)**, 38-44.

Koojiman, S., (2000): Dynamic energy and mass budgets in biological systems. Cambridge, United Kingdom: Cambridge University Press.

Kouwenberg, J. H. M.; Browman, H.; Cullen, J. J.; Davis, R. F.; St. Pierre, J. F.; Runge, J. A., (1999): Biological weighting of ultraviolet (280-400 nm) induced mortality in marine zooplankton and fish. I Atlantic cod (*Gadhus morhua*) eggs. *Marine Biology* **134**, 269-284.

Leech, D. M.; Williamson, C. E., (2000): Is tolerance to UV radiation in zooplankton related to body size, taxon, or lake transparency? *Ecol. Appl.* **10**, 1530-1540.

Losey, G. S.; Hydes, D., (1998): The UV visual world of fishes. *J. Fish Biol.* **54**, 921–945.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J., (1951): Protein measurement with the Folin phenol reagent. *J. Biol. Chem* **41**, 1863-1870.

Ma, Z.; Li, W.; Gao, K., (2010): Horizontal migration of Acartica pacifica Steuer (copepoda) in response to UV-radiation. *J. of Photoch. and Photobio. B: Biology* **101**, 233–237.

Ma, Z.; Li, W.; Gao, K., (2012): Impacts of solar UV radiation on grazing and lipids oxidation of Acartia pacifica Steuer (Copepod). *Acta Oceanologica Sinica* **31:**, 126–134.

Ma, Z.; Li, W.; Gao, K., (2013): Impacts of UV radiation on respiration, ammonia excretion and survival of copepods with different feeding habits. *Hydrobiologia* **701**, 209–218.

MacFadyen, E. J.; Williamson, C. E.; Grad, G., (2004): Molecular response to climate change: temperature dependence of UV-induced DNA damage and repair in the freshwater crustacean Daphnia pulicaria. *Global Change Biol.* **10**, 408–416.

Mattie, M. D.; Freedman, J. H., (2001): Protective effects of aspirin and vitamin E (alpha-tocopherol) against copper and cadmium-induced toxicity. *Biochem. Biophy. Res. Com.* **285**, 921–925.

Menze, M. A.; Fortner, G.; Nag, S.; Hand, S. C., (2010): Mechanisms of apoptosis in Crustacea: what conditions induce versus suppress cell death? *Apoptosis* **15**, 293–312.

Meyer-Rochow, V. B., (2000): Risks, especially for the eye, emanating from the rise of solar UV-radiation in the Arctic and Antarctic regions. *Int. J. Circumpolar Health* **59**, 38–51.

Misra, H. P.; Fridovich, I., (1972): The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem* **247**.

Moeller, R. E.; Gilroy, S.; Williamson, C. E.; Grad , G.; Sommaruga, R., (2005): Dietary acquisition of photoprotective compounds (mycosporine-like amino acids, carotenoids) and acclimation to ultraviolet radiation in a freshwater copepod. *Limnology and Oceanography* **50**, 427-439.

Norval, M.; Cullen, A. P.; de Gruijl, F. R.; Longstreth, J.; Takizawa, Y.; Lucas, R. M.; Noonan, F. P.; van der Leun, J. C., (2007): The effects on human health from stratospheric ozone depletion and its interactions with climate change. *Photochem. Photobiol. Sci.* **6** 232–251.

Ohkawa, H.; Ohishi, N.; Yagi, K., (1979): Assay for lipid peroxides in animal tissue by thiobarbaturic acid reaction. *Anal. Biochem* **95**, 351–358.

Ozmen, I.; Bayır, A.; Cengiz, M.; Sirkecioglu, A. N.; Atamanalp, M., (2004): Effects of water reuse system on antioxidant enzymes of rainbow trout (Oncorhynchus mykiss W., 1792). *Vet Med* **49**, 373–378.

Paunel, A. N.; Dejam, A.; Thelen, S.; Kirsch, M.; Horstjann, M.; Gharini, P., (2005): Enzyme-independent nitric oxide formation during UVA challenge of human skin:characterization, molecular sources, and mechanisms. *Free Radic. Biol. Med*. **38**, 606-615.

Powell, S. R., (2000): The antioxidant properties of zinc. *J. Nutr.* **130**, 1447–1454.

Qu, Y.; Feng, H.; Wang, Y.; Zhang, M.; Cheng, J.; Wang, X.; An, L., (2006): Nitric oxide functions as a signal in ultraviolet-B induced inhibition of pea stems elongation. . *Plant Science* **170**, 994–1000.

Rajkumar, J. S. I.; John Milton, M. C.; Arockia Rita, J. J., (2011): Lipid peroxidation and reduced glutathione as a biomarker in common clam, Meriterix meriterix to shor term chronic copper exposure. *Asian J. of Sci. and Techno.* **2**, 009-012.

Rautio, M.; Korhola, A., (2002): Effects of ultraviolet radiation and dissolved organic carbon on the survival of subarctic zooplankton. *Polar Biol.* **25**, 460–468.

Regoli, F.; Gorbi, S.; Frenzilli, G.; Nigro, M.; Corsi, I.; Focardi, S.; Winston, G. W., (2002a): Oxidative stress in ecotoxicology: from the analysis of individual antioxidants to a more integrated approach. *Mar. Environ. Res.* **54**, 419–423.

Regoli, F.; Pellegrini, D.; Winston, G. W.; Gorbi, S.; Giuliani, S.; Virno-Lamberti, C.; Bompadre, S., (2002b): Application of biomarkers for assessing the biological impact of dredged materials in the Mediterranean: the relationship between antioxidant responses and susceptibility to oxidative stress in the red mullet (*Mullus barbatus*). *Mar.Pollut. Bull.* **44**, 912-922.

Rhode, S. C.; Pawlowski, M.; Tollrian, R., (2001): The impact of ultraviolet radiation on the vertical distribution of zooplankton of the genus Daphnia. *Nature* **412**, 69–72.

Rijstenbil, J. W., (2000): Effects of periodic, low UVA radiation on cell characteristics and oxidative stress in the marine planktonic diatom Ditylum brightwellii. *Eur. J. Phycol.* **36**, 1-8.

Rocco, C.; Oppezzo, O.; Pizarro, R.; Sommaruga, R.; Ferraro, M.; Zagarese, H., (2001): Ultraviolet damage and counteracting mechanisms in the freshwater copepod *Boeckella poppei* from the Antarctic peninsula. *Limnology and Oceanography* **47**, 829–836.

Romero-Graillet, C.; Aberdam, E.; Biagoli, N.; Massabni, W.; Ortonne, J. P.; Ballotti, R., (1996): Ultraviolet B radiation acts through the nitric oxide and cGMP signal transduction pathway to stimulate melanogenesis in human melanocytes. *J. Biol. Chem.* **271**, 28052–28056.

Sancho, E.; Villarroel, M. J.; Andreu, E.; Ferrando, M. D., (2009): Disturbances in energy metabolism of Daphnia magna after exposure to tebuconazole. *Chemosphere* **74**, 1171–1178.

Scott, J. D.; Chalker-Scott, L.; Foreman, A. E.; D'Angelo, M., (1999): Daphnia pulex fed UVB-irradiated *Chlamydomonas reinhardtii* show decreased survival and fecundity. *Photochemistry and Photobiology* **70**, 308-313.

Seo, S.; Choi, H.; Chung, H.; Hong, C., (2002): Time course of expression of mRNA of inducible nitric oxide synthase and generation of nitric oxide by ultraviolet B in keratinocyte cell lines. . *British Journal of Dermatology* **147**, 655–662.

Sommaruga, R.; Garcia-Pichel, F., (1999): UV-absorbing mycosporine- like compounds in planktonic and benthic organisms from a high-mountain lake. . *Archiv fu¨r Hydrobiologie* **144**, 255–269.

Stephensen, E.; Svavarsson, J.; Sturve, J.; Ericson, G.; Adolfsson-Erici, M.; Forlin, L., (2000): Biochemical indicators of pollution exposure in shorthorn sculpin (*Myoxocephalus scorpius*), caught in four harbours on the southwest coast of Iceland. *Aquat. Toxicol.* **48**, 431–442.

Tartarotti, B.; Baffico, G.; Temporetti, P.; Zagarese, H. E., (2004): Mycosporine-like amino acids in planktonic organisms living under different UV exposure conditions in Patagonian lakes. *J. of Plankton Research* **26(7)**, 753–762 .

Vega, M. P.; Pizarro, R. A., (2000): Oxidative stress and defence mechanisms of the freshwater cladoceran Daphnia longispina exposed to UV radiation. *J. Photochem. Photobiol. B: Biol.* **54**, 121-125.

Villarroel, M. J.; Sancho, E.; Andreu-Moliner, E.; Ferrando, M. D., (2009): Biochemical stress response in tetradifon exposed Daphnia magna and its relationship to individual growth and reproduction. . *Science of the Total Environment* **407** 5537–5542.

Vlahogianni, T.; Dassenakis, M.; Scoullos, M. J.; Valavanidis, A., (2007): Integrated use of biomarkers (superoxide dismutase, catalase and lipid peroxidation) in mussels *Mytilus galloprovincialis* for assessing heavy metals' pollution in coastal areas from the Saronikos Gulf of Greece. *Mar Pol Bul* **54**, 1361–1371.

Willis, K. J.; Ling, N.; Ann Chapman, M., (1995): Effects of temperature and chemical formulation on the acute toxicity of pentachlorophenol to Simocephalus vetulus (Schoedler, 1858) (Crustacea: Cladocera). *New Zealand Journal of Marine and Freshwater Research* **29**, 289-294.

Wold, A.; Norrbin, F., (2004): Vertical migration as a response to UVR stress in Calanus finmarchicus female and nauplii. *Polar Research* **23**, 27–34.

Xue, L.; Li, S.; Sheng, H.; Feng, H.; Xu, S., (2007): Nitric oxide alleviates oxidative damage induced by enhanced ultraviolet-B radiation in cyanobacterium. *Current Microbiology* **55**, 294–301.

Yildirim, N. C.; Benzer, F.; Danabas, D., (2011): Evaluation of environmental pollution at Munzur River of Tunceli applying oxidative stress biomarkers in Capoeta trutta (Heckel, 1843). *J. Anim. Plant. Sci.* **21(1)**, 66–71.

Yu, J.; Yang, G.; Tian, J., (2009): Effects of UV-B radiation on ingestion, fecundity, population dynamics and antioxidant enzyme activities of Schmackeria inopinus (Copepoda Calanoida). *Journal of Experimental Marine Biology and Ecology* **381** 74–81.

Zhang, J.; Shen, H.; Wang, X.; Wu, J.; Xue, Y., (2004): Effects of chronic exposure of 2,4-dichlorophenol on the antioxidant system in liver of freshwater fish Carassius auratus. *Chemosphere* **55**, 167–174.