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UV radiation effects and bioaccumulation of UV-absorbing compounds in *Artemia persimilis* larvae

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Abstract. The aims of this study were to determine if larvae of *Artemia persimilis* (Crustacea: Branchiopoda) can bioaccumulate ultraviolet radiation (UVR) absorbing compounds like mycosporine-like aminoacids (MAAs) through the diet and the probable protective role of this compounds on larvae of shrimp. Metanauplii II-VII were feeding with microalgae to produce UV absorbing compounds like *Thalassiosira fluviatilis* and *Chaetoceros gracilis* previously irradiated with UVR. Additionally, were studied if this irradiation affects their ingestion rate, survival and growth. Microalgae grown in F/2 media during 20 days in semi-continuous cultures, under two radiation treatments: 1) PAR (photosynthetically active radiation) (400-700nm) and 2) UVR+PAR (280-700 nm). Only for those specimens fed with UVR-irradiated algae, a peak of absorption (334 nm ~ 0.13 OD/n) in the UVR range was observed. There were no significant differences in Ingestion rate (192 ±10 and 176±7 µL ind^{-1} h^{-1}) and growth (400 y 370 %) between no irradiated and UV-irradiated treatment, respectively. After 48 hours UVR stress exposition mortality rates were ~50 % for individuals fed PAR-treated algae; whereas those fed UVR-irradiated algae present survival rates ~90%. The results implies that larval stages of *A. persimilis* can bioaccumulate UV-absorbing compounds through their diets, supporting the idea that this compounds plays an important role by protecting against UVR stress.

Keywords: crustacean larvae, UV-induced stress, bioaccumulation, photoprotection

Resumen. Efectos de radiación UV y bioacumulación de compuestos que absorben UV en *Artemia persimilis.* El objetivo del presente estudio fue determinar si larvas de *Artemia persimilis* (Crustacea, Branchiopoda) son capaces de bioacumular compuestos que absorben radiación ultravioleta (RUV), como micosporinas "like"aminoácidos (MAAs) a partir de la dieta y el posible efecto protector de dichos compuestos. Los metanauplios II-VII se alimentaron con microalgas que producen compuestos que absorben UV como *Thalassiosira fluviatilis* y *Chaetoceros gracilis* previamente irradiadas con RUV. Se estudió además si esta radiación afecta la tasa de ingestión, supervivencia y crecimiento. Las microalgas crecieron en medio f/2 durante 20 días en cultivos semi-continuos bajo dos tratamientos de radiación: 1) PAR (radiación fotosintéticamente activa) (400-700nm) y 2) RUV+PAR (280-700nm). Sólo en los especimenes alimentados con algas UV-irradiadas se observó un pico de absorbancia en el rango de RUV (334 nm ~ 0.13 OD/n). No se encontraron diferencias significativas en la tasa de ingestión (192±10 y 176±7 µL ind^{-1} h^{-1}) y crecimiento (400 y 370%) de larvas alimentadas con fitoplancton no irradiado e irradiado con RUV, respectivamente. Luego de 48 h de estrés por RUV los metanauplios alimentados con algas PAR-irradiadas, mostraron una mortalidad del 50%, mientras que aquellos alimentados con algas UV- irradiadas presentaron supervivencias del 90%. En base a los resultados podemos concluir que los estados larvales de *A. persimilis*

pueden bioacumular compuestos que absorben UV a través de su dieta, sugiriendo que estos compuestos juegan un importante rol en la protección ante un estrés por RUV.

Palabras clave: larva de crustáceo, estrés UV-inducido, bioacumulación, fotoprotección

Introduction

The Brine shrimp *Artemia* is a worldwide crustacean adapted to live in stressful environmental conditions of hypersaline habitats such as saline lakes and ponds in all continents with the exception of Antarctica (Ogello et al. 2014). In the New World, the genus is represented by the species *Artemia franciscana* Kellogg, 1906, and *A. persimilis* Piccinelli & Prosdocimi, 1968 (Gajardo et al. 2002, Cohen 2012). *A. persimilis*, is geographically restricted to southern South America, from approximately 36-37°S to higher latitudes, in Argentina and Chile (Cohen 2012); it is well known for its ability of adaptation to diverse biotopes (inland salt lakes, coastal lagoons and solar salt pools) at variable salinity, light and temperature. This genus is commercially exploited in its larval form and as an adult, chiefly for aquaculture purposes. As natural and live food for larvae, juveniles, adults and breeding shrimp and fish, is irreplaceable, for its massive energy reserves, and in some cases due to the profile of the polyunsaturated fatty acids (Guermazi et al. 2008). Ultraviolet radiation (UVR-280-400 nm) is a natural component of solar radiation; therefore the aquatic environment has always been exposed to UVR (Häder et al. 2011). However, in recent years, anthropogenic influence via stratospheric ozone depletion has caused an increase in the UVR flux to the earth surface (McKenzie et al. 2010). In this way, the effects of enhanced UVR on the environment (both primary producers and their consumers) can be considered a new problem (Whitehead et al. 2001, Häder et al. 2011). Changes in phytoplankton composition could have an adverse effect on the quality of food available to the zooplankton. UVR can reduce phytoplankton photosynthesis, producing changes in its specific composition and can alter the nutritious quality of phytoplankton (Newman et al. 2000, Beardall et al. 2009, Finkel et al. 2010, Nahon et al. 2010). This fact promotes indirect adverse impacts on secondary producers through the food web (Przeslawski 2005, Tartarotti & Torres 2009).

Terrestrial, marine and freshwater organisms have developed strategies to diminish the direct and indirect damaging effects of environmental UVR by synthesizing, accumulating and metabolizing a variety of UV-absorbing compounds called mycosporine-like amino acids [MAAs] (Shick & Dunlap 2002, Carreto & Carignan 2011). Considerable interest has been centered on MAAs since experimental evidence indicates that in marine organisms the major functions of MAAs are to act as active UV filters (Moeller et al. 2005, Carreto & Carignan 2011, Rastogi et al 2010) and/or as antioxidants (Shick & Dunlap 2002, Arbeloa et al. 2010, Figueroa et al. 2011). These photoprotective compounds are common in some species of microalgae such as Bacillariophyceae, Dinophyceae and Primnesiophyceae (Carreto & Carignan 2011) and macroalgae like Rodophyceae (Rastogi et al. 2010, Ha et al. 2012); moreover, they have also been found in vertebrates and invertebrates. Animals incorporate MAAs either through the diet (Newman et al. 2000, Riemer et al. 2007) or as a symbiotic association with microalgae (Banaszack et al. 2006) as a means of acquiring some protection against UVR (Whitehead et al.2001, Zengling et al. 2012, Riemer et al. 2007). Other hypotheses about the role of MAAs in biological systems have been formulated: they have influence on reproduction of marine invertebrates (Adams et al. 2001) and may play a role under desiccation or thermal stress in certain organisms (Oren & Gunde-Cimerman 2007, Yoshik et al. 2009). Finally, MAAs can act as an intracellular nitrogen reservoir (Korbee-Peinado et al. 2004). Recently, Kicklighter et al. (2011) showed that pyrimidines and MAAs work as alarm cues in the defensive secretions of the sea hare *Aplysia californica.*

Since there are not studies on *A. persimilis*, the aims of this research were to determine if larvae of this species can bioaccumulate UV-absorbing compounds through a diet of UV-irradiated microalgae and asses if this irradiation affects their survival and growth. Additionally, the protective role of UV-absorbing compounds on larvae UV-stressed was studied. We chose *T. fluviatilis* and *C. gracilis* as the dietary algae because they have a high nutritional value, provide vitamins and polyunsaturated fatty acids, and therefore are of common use in aquaculture practices (Støttrup et al. 1999, Mallo & Fenucci 2004). Moreover, it is known that these species produce UV-absorbing compounds (OD: 334) when are exposed to UVR (Marcoval et al. 2007, Häder et al. 2011)

Materials and Methods

Experimental design consisted in three stages: 1) Culture preparation algae with presence of UV absorbing compounds, diatoms *Chaetoceros gracilis* and *Thalassiosira fluviatilis*) were previously grew under two radiation regimes: (a) Photosintetically Active Radiation, PAR (400-700 nm) and (b) UVR+PAR (280-700 nm) under irradiance of 300 µmol quanta m^{-2} s^{-1} for photo synthetically active radiation (PAR -Lutron Lx. 107) and 20 W m^{-2} -Lutron UVA 340- simulating natural conditions

2) Feeding early planktonic stages of *A. persimilis* with the two microalgae UVR –MAAs induced cultures (diatoms *C. gracilis* and *T. fluviatilis*) previously grew under two radiation regimes: (see point 1) After feeding with these diets, ingestion rates, survival and growth of zooplankter were determined.

3) By the end of the feeding experiment, larvae of *A. persimilis* were subject to UVR stress (20 W m^{-2}) for 48 h, after that, survival and content of UV–absorbing compounds were estimated for larvae fed PAR and UVR+PAR-irradiated phytoplankton.

1. Phytoplankton culture: C. gracilis and *T. fluviatilis* were grown in a semi-continuous batch cultures (Hoff and Snell, 2001) in f/2 medium (Guillard and Ryther, 1962) with autoclaved, 0.22-µm filtered seawater (S =33) at 20-22 ºC, using 1-L UV-transparent polycarbonate containers (Plexiglas UVT, GS 2458, Röhm and Haas, Darmstadt, Germany). Light sources were 40W cool-white fluorescent bulbs (Philips) for PAR, and Q-Panel UVA-340 bulbs (for UV) placed 20 cm from water surface; photoperiod was set at a 12:12 h, with an average irradiance of 300 µmol quanta m^{-2} s^{-1} for PAR (Lutron Lx. 107) and 20 W m^{-2} for UVR (Lutron UVA 340) simulating natural conditions Two irradiation treatments were performed: a) PAR, in which containers were externally covered with Ultraphan film (UV Opak, Digefra; 50% transmission at 395 nm), and b) UVR+PAR, in which containers received UVR (280-400 nm) + PAR (400-700 nm).

For the experiments, microalgae were harvested at the stationary phase during 20 days (Table I) culturing in order to have significantly higher content of UV- absorbing compounds (Neale et al. 1998, Zudaire & Roy 2001) which were determined by spectrophotometry along the time.

2. Feeding experiments on Artemia persimilis cultures: Nauplii of *A. persimilis* were hatched from dried eggs obtained from Salinas Chicas, (38°44' S, 62°57' W, Buenos Aires, Argentina).Cysts were de-capsulated in a bleach solution (Lavens & Sorgeloos 2000). First stage nauplii (NI) hatched after 24 h; and were transferred to 6 L containers filled with 0.45 µm filtered seawater (S =33), for 48 h, until they reached the next naupliar stage, metanauplii II (MNII), which was used in experiments.

2a. Mortality –feeding 48 h experiments: MNII (n=300) were placed under light continues with an average irradiance of 300 µmol quanta m^{-2} s^{-1} for PAR (Lutron Lx. 107), and fed daily with mix of phytoplankton species (*C. gracilis* and *T. fluviatilis* at a ratio of 1:1) density 5 to 10 × 10^4 cells mL^{-1} (Isiordia-Perez et al. 2006) previously irradiated with two treatments (i.e. 2×2 design) as show Table I. All treatments were run by triplicate.

2b. Growth and development: Every 24 h, 5 individuals were taken from each treatment and preserved in alcohol 70% for subsequent measurement. Length was considered from the rostrum to the telson. Development of organisms was determined based on the work of Johnson &Olson (1948).

Table I. Experimental design for feeding experiment.

Food Treatment	Food Item	Food Irradiance treatment	Deeply cell density cells mL^{-1}	Metanaupli n
PAR	*Thalassiosira fluviatilis* *Chaetoceros gracilis*	300 µmol quanta m^{-2} s^{-1} for PAR (Lutron Lx. 107)	5-10 x 10^4	300
PAR + UVR	*Thalassiosira fluviatilis* *Chaetoceros gracilis*	300 µmol quanta m^{-2} s^{-1} for PAR (Lutron Lx. 107) and 20 W m^{-2} for UVR (Lutron UVA 340)	5-10 x 10^4	300

Food treatment imposed on the food item in the experimental design: Food Irradiance treatment, deeply cell density and number of metanaupli used. PAR: photosynthetic active radiation, UVR: ultraviolet radiation.

2c. Ingestion rate (Ir): Each nauplii´s ingestion rate (n=10, at three different times) was calculated during and the end of the feeding experiment, based on the formula:

$$I_r = (Ci-Cf) / n * t$$ (Båmstedt 2000).

Where, Ci = Initial concentration of phytoplankton in cell number; Cf = final concentration of cell number of phytoplankton, n = number of *Artemia* nauplii and t = time.

3. Survival: living organisms were counted at the beginning and at the end of the feeding experiment, considering dead individuals, those with total absence of mobility in their locomotors appendages. After UVB stress exposure, two replicates were made from each feeding treatment, thus obtaining the final percentage of survival.

4. UV absorbing compounds Absorbance Spectra

a) Phytoplankton cultures: Samples (20-100 mL depending on the density of phytoplankton) were concentrated on Whatman GF / F glass fiber (25mm) daily. Photosynthetic compounds and UV-absorbing compounds were extracted in 7 mL of absolute methanol and subsequently centrifuged for 15 minutes at 2000 rpm. Concentrations of UV-absorbing compounds were estimated with a spectrophotometer Hewlett Packard HP model 8453E, scanning between 250-750 nm. It is known that there is a good relationship between the specific absorption at 320-340 nm and the amount of UV-absorbing compounds e.g. MAAs biomass (Dunlap et al. 1995, Hernández-Moresino et al. 2014). By using Origin Pro program, concentration of chlorophyll and UV-absorbing compounds were calculated from peak heights absorbance (420-440nm and 665nm) and (310-360nm) respectively, divided as a function of the number of cells in the culture at the time of sampling.

b) Nauplii: For extraction of UV-absorbing compounds, after the experiments larvae were placed in 5 ml of absolute methanol at 4°C for at least 4 h during this period, individuals were macerated with a glass rod. After extraction, samples were centrifuged 15 min. at 2000 rpm. Concentration of UV- absorbing compounds (Gonçalves et al. 2010) were estimated using a scanning spectrophotometer between 250-750 nm (Hewlett Packard. Model-HP-8452A) and the program Origin Pro, as for the phytoplankton, from peak heights absorbance (310-360 nm), although in this case divided by the number of individuals.

5. Statistical analysis: Data of longitude of zooplankter were subjected to normality tests by Lilliefors and homoscedasticity of variances by Bartlett, the significance of the treatments were compared by ANOVA two-ways and with Tukey's multiple range test (Zar 1999). The Chi^2 test was run to determine significance of survival. All analyzes were performed with the Statistical Package Statistix 8 (version 2000), at a significant level of $\alpha = 0.05$.

Results

Phytoplankton cultures: Mean sizes of both diatoms *C. gracilis* and *T. fluviatilis* were 6 ± 0.5 µm x 4 ± 0.5 µm. Maximum concentration values in cells mL^{-1}reached by the species *T. fluviatilis* and *C. gracilis*, with maximum absorbance peaks for UV-absorbing compounds, are presented in Table II. The diatoms used like food: *T. fluviatilis* and *C. gracilis,* show significant differences in growth curves rates for PAR and PAR+UVR radiation treatments (ANOVA, $p<0.05$) Of the two species irradiated, *T. fluviatilis* first reached the exponential phase. *C. gracilis* showed higher values of absorbance peak for UV-absorbing compounds ($p<0.05$) during the induction period, with significant differences between radiation treatments.

Mortality –feeding experiments on Artemia persimilis cultures: The results of two-way ANOVA revealed that there was no significant differences in growth of individuals fed with both mix phytoplankton cultures no irradiated (PAR) and (previously irradiated (PAR and PAR+UVR).

Table II. Phytoplankton species used like food. Maximum cells concentration reached in cell mL^{-1} ± standard deviation (SD); days of exposition to ultraviolet radiation (UVR); maximum UVR absorbance peak (nm) reached during growth; maximum concentration of UV absorbing compounds expressed in optical dioptry cells mL^{-1} ± SD. The superscripts indicate significant differences (ANOVA $p< 0.05$).

Phytoplankton spp. like food	Cell mL^{-1} ± SD		Days of exposition to UVR	Absorbance UVR Peak (nm)	[UV absorbing compounds] (OD cells mL^{-1}) ± SD	
	PAR	PAR+UVR			PAR	PAR+UVR
Thalassiosira fluviatilis	863,000 ±12,940 [i]	570,000 ±21,920 [f]	12	334	$1.6.10^{-8}$ ±1.1 10^{-9} [a]	$4.2.\ 10^{-8}$ ±5.9 10^{-10} [b]
Chaetoceros gracilis	186,000 ±1,438 [g]	105,000 ±1,827 [e]	15	334	$1.53.\ 10^{-8}$ ±2.9. 10^{-9} [a]	$8.12.\ 10^{-7}$ ±1.5. 10^{-7} [c]

Naupliar NI stages with initial average lengths of 0.5 mm, reached at the end of the feeding experiment maximum values of 4.11 and 3.72 mm for treatments PAR and PAR + UVR, respectively (Fig. 1).

Ingestion rates were no significant differences, with values of 192 ±10 and 176±7 µL ind^{-1} h^{-1} for treatments PAR and PAR + UVR, respectively. Related to concentration of UV-absorbing compounds, peaks of maximum absorbance were found at 334nm. Larvae after 6 days of culture showed a value of 0.132 OD/n [n=20] for the treatment PAR + UVR, while individuals fed with non- UV-irradiated algae, kept very low concentrations ≤0.05 OD/n [n=20] (Fig. 2). Survivals reached average values ~90 % (ANOVA $p<0.05$), with no significant differences between treatments (Fig. 3).

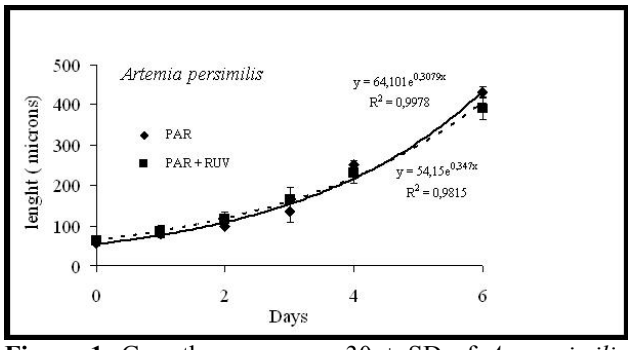

Figure 1. Growth average n= 30 ± SD of *A. persimilis* during 6 days feeding experiments with phytoplankton species: *T fluviatilis and C. gracilis*. The symbols indicate the different radiation treatments imposed on the food: diamond photosynthetic active radiation (PAR), square PAR + Ultraviolet radiation (UVR).

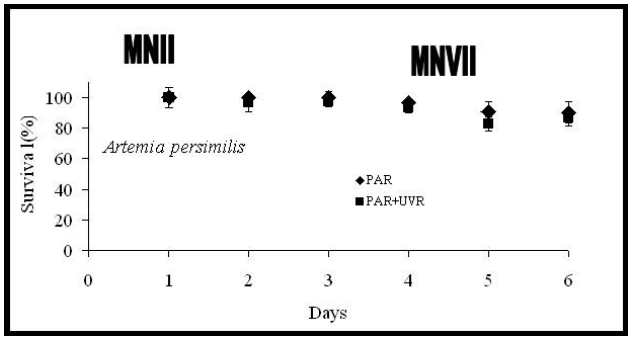

Figure 2. Concentration average of UV- absorbing compounds during 6 days feeding experiments in *A. persimilis*. The symbols indicate the different radiation treatments imposed on the food: diamond photosynthetic active radiation (PAR), square PAR + Ultraviolet radiation (UVR). Day 8 shows concentration of UV-absorbing compounds after 48 h starving and UV-stress period of *A. persimilis.* The superscripts a, b, c, d indicates significant differences (ANOVA $p< 0.05$).

After the fasting period of 48 h (day 8) under UV-irradiation stress, *Artemia* metanauplii fed with UV-irradiated algae presented an absorbance peak at 334 nm ~ 0.1 OD/n [n=200], which was significantly higher ($p<0.05$) than absorbance found in the animals fed only with PAR- irradiated algae, with a peak 334 nm closely to 0 OD/n [n=90] (Fig. 2). During the period of stress, significant differences in survival were observed (ANOVA $p \leq 0.05$) between feeding treatments: values ~90 % for *A. persimilis* larvae previously fed with UV-irradiated algae, and values ~53% on larvae fed with algae PAR irradiated (Fig. 4).

Discussion and Conclusions

Previous aquaculture studies on *Artemia* have shown very good survival in early stages of development by feeding with groups of algae Bacillarophyceae and Chlorophyceae (Liang et al. 2006).. In this work, the concentrations of UV absorbing compounds in the microalgae reached values between 4.2 and 8.10^{-8} OD cells mL^{-1} on UV-treatments, similar results were found by Marcoval et al. (2007) were *C. gracilis* and *T. fluviatilis* increased significantly the concentration of UV-absorbing compounds throughout the experiment.

The response of *A. persimilis* to the presence of UVR and PAR were studied at levels corresponding to local noontime solar radiation of sunny days during mid-summer. No significant differences in Ingestion rate and growth of individuals of *A. persimilis* between radiation treatments could indicate that UV does not modify nutritious capacity from food source (Diatoms) and IR could directly proportional to phytoplankton cells number but no relationship was found with presence/absence of UV-absorbing compounds in algae cultures (ANOVA $p<0.05$). Similar results were found in Antarctic krill (*Euphausia superba*) when individuals survived during 38 days, fed with the diatom *Phaeocystis antarctica* that was grown under PAR+UVR regime (Newman et al. 2000). Other works were performed in crabs (Hernández-Moresino & Helbling 2010, Hernández-Moresino et al. 2014) and copepods (Moeller et al. 2005) with analogous conclusions.

Regarding to bioaccumulation of UVR absorbing compounds, there are evidences of transfer of UV- absorbing compounds (e.g. MAAs) from primary producers to higher levels through the food chain (e.g. sea hares Kicklighter et al., 2011). Some studies on Antarctic invertebrates revealed

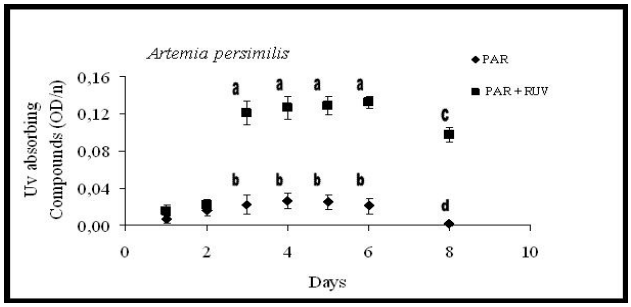

Figure 3. Survival average of *A. persimilis* during 6 days of feeding experiments from metanauplii II (MNII) to metanauplii VII (MNVII). The symbols indicate the different radiation treatments imposed on the food: diamond photosynthetic active radiation (PAR), square PAR + Ultraviolet radiation (UVR).

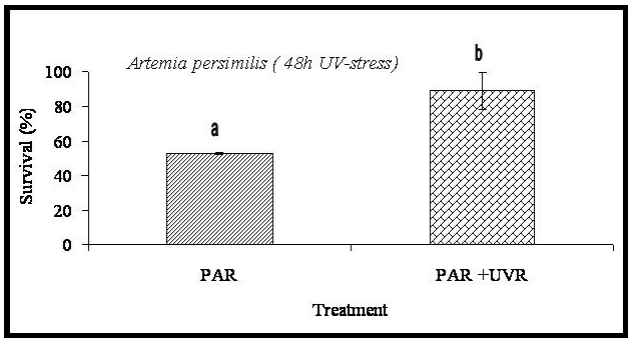

Figure 4. Final survival average (n=90 ± SD) of *A. persimilis* (at day 8 of experiment, after 48 h starving and exposed to UV-stress period of Q-panel UV-340 lamp. Initials indicate the different radiation treatments imposed on the previous food: photosynthetic active radiation (PAR), ultraviolet radiation (UVR). The superscripts indicate significant differences (ANOVA $p < 0.05$).

bioaccumulation of MAAs from a diet of macroalgae and diatoms (Mc Clinctock & Karentz 1997). Other study on amphipods fed with red seaweed (Helbling et al. 2002), reported similar results in the accumulation of UV compounds through the diet, since amphipods that received algae with UV- absorbing compounds, were more resistant to UVR, than those fed algae without compounds. Our results showed that metanauplii of *A. persimilis* improve its survival and have an enhancement of UV-absorbing compounds when they were feeding by a mix of UV- irradiated *T. fluviatilis* and *C. gracilis* until 6 days after hatching, and these compounds may offer protection against moderate UV-radiation stress. Furthermore metanauplii of this species fed with UV-irradiated diatoms, showed mortality of 10% when were exposed to moderate UVR during 48 h. In contrast, in metanauplii fed on no UV-irradiated algae, mortality was 50%. Whitehead & Vernet (2000) demonstrated that UV-absorbing compounds offer protection to UV radiation, even when these UV-absorbing compounds are present in low concentrations. Other study with freshwater copepods showed that the presence of UV-absorbing compounds decreases mortality rate in the copepod *Metacyclops mendocinus* when subjected to UVR stress (Gonçalves et al. 2002).

Finally, UVR is an important mutagen, changing the molecular structure of DNA consequently, the biota of shallow aquatic habitats lacking photoprotective strategies to avoid or repair the damage caused by exposure to UVR, may be particularly susceptible to UVR-induced damage (Friedberg 2003). Since brine shrimp have many advantages for use in aquaculture and genetics works (Gajardo & Beardmore 2012; Khairi & El-Sayed 2012), different lines of study on the effects of UVR on brine shrimp are likely to produce useful results, for example to compare effects of UVR on survival rate of different zooplankton species in Argentina using *A. persimilis* as control.

Understanding the mechanism of adaptation to stressful environments in *Artemia* has some indirect benefits to humans, because of the role it plays in cultures of marine fish and crustaceans (Dhont & Sorgeloos 2002; Khairi & El-Sayed 2012). Larval stages of some of these species need live diets as first feed. *Artemia* larvae (which can be nutritionally enhanced) provide not only basic nutritional requirements but also enzymes and other valuable dietary elements that make it an attractive prey for predatory fish larvae. The nutritional value of the adult *Artemia*, is higher than that of the newly hatched nauplii; nauplius protein content is 47% and 60% in adults The adult brine shrimp contains high amount of digestive enzymes, which can improve digestion of predators (Cisneros &Vinatea 2009).

Furthermore, production of *Artemia* is being gradually spreading globally, in small and large scale, given its high demand and severe shortages due to the depletion of natural sources. Its high cost and low supply available increase the cost of massive larval production in laboratories and aquaculture as an effective alternative in these production centers. Since *Artemia* is the main food for early stages in culture of crustacean penaeoids, a diet based on adult enriched *Artemia*, could induce the development and maturation of the shrimp. Also, could be useful study if UV-absorbing compounds can be transfer to the trophic chain and offer protection to postlarvae of these organisms when they are growing in shallow ponds that have a maximum depth between 1-15 m (Mallo et al. 1999,

Thakur & Lin 2003) and UVR penetrates to the bottom of the water column (Villafañe et al.2003).

The results implies that larval stages (MNI and MNII) of *A. persimilis* can bioaccumulate UV-absorbing compounds through their diets, supporting the idea that this compounds plays an important role by protecting against UVR stress.. These results highlight the importance of these compounds in food chains since organisms in higher levels of the food chain could absorb them, and thus provide resistance to increased levels of UVR.

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References

Adams, N. L., Shick, J. M. & Dunlap, W. C. 2001. Selective accumulation of mycosporine-like amino acids in ovaries of the green sea urchin *Strongylocentrotus droebachiensis* is not affected by ultraviolet radiation. **Marine Biology**, 138: 281–294.

Arbeloa, E. M., Uez, M. J., Bertolotti, S. G. & Churio, M. S. 2010. Antioxidant activity of gadusol and occurrence in fish roes from Argentine Sea. **Food Chemistry**, 119: 586–591.

Bamstedt, U. 2000. Life cycle, seasonal vertical distribution and feeding of *Calanus finmarchicus* in Skagerrak coastal water. **Marine Biology**, 37: 279-289.

Banaszak, A. T., Barba Santos, M. G., LaJeunesse, T. C., Lesser, M. P. 2006. The distribution of mycosporine-like amino acids (MAAs) and the phylogenetic identity of symbiotic dinoflagellates in cnidarian hosts from the Mexican Caribbean. **Journal of Experimental Marine Biology and Ecology**, 337:131–146.

Beardall, J., Allen, D., Bragg, J. et al. 2009. Allometry and stoichiometry of unicellular, colonial and multicellular phytoplankton. **New Phytologist**, 181: 295–309.

Carreto, J. I. & Carignan, O. M. 2011. Mycosporine-like amino acids: Relevant secondary metabolites. Chemical and ecological aspects. **Marine Drugs**, 9: 387–446.

Cisneros, C. & Vinatea, E. 2009. Producción de biomasa de *Artemia franciscana* (Kellog, 1906) utilizando diferentes dietas. **Ecological Applications**, 8: 9-14.

Cohen, R. G. 2012. Review of the biogeography of *Artemia* Leach 1819 (Crustacea: Anostraca) in Argentina. **International Journal of Artemia Biology**, 2: 9-23.

Dhont, J. & Sorgeloos, P. 2002. "Applications of *Artemia*". Pp 251–277. *In*: Abatzopoulos, T. J., Beardmore, J. A., Clegg, J. S. & Sorgeloos, P. (Eds.). **Artemia: Basic and Applied Biology** Dordrecht: Kluwer Academic Publishers.

Dunlap, W. C., Rae, G. A., Helbling, E. W., Villafañe, V. E. & Holm-Hansen, O. 1995. Ultraviolet-absorbing compounds in natural assemblages of Antarctic phytoplankton. **Antarctic Journal of the United States**, 30: 323–326.

Figueroa, F. L., Korbee, N., De la Coba, F., Gil, C., Bonomi, J., Güenaga L, Gómez, J. L., Abreu, M. H., Pereira, R., Sousa-Pinto, I. 2011. Valorización de la biomasa de macroalgas en acuicultura multitrófica integrada (AMTI): Aplicaciones en cosmética y nutraceutica (fotoprotectores y antioxidantes). Pp. 111-137. *In*: Vázquez, U., Incera, M., Fernández, R., Maroto, J. (Eds). **Macroalgas en la Acuicultura Multitrófica Integrada Peninsular**. Centro Tecnológico del Mar, Vigo, Spain, 176 p.

Finkel, Z. V., Beardall, J., Flynn, K. J., Quigg, A., Rees, T. A. V. & Raven, J. A. 2010. Phytoplankton in a changing world: cell size and elemental stoichiometry. **Journal of Plankton Research**, 32: 119–137.

Friedberg, E. C., 2003. DNA damage and repair. **Nature**, 421(6921): 436-440.

Gajardo, G., Abatzopoulos, T. J., Kappas, I. & Beardmore, J. 2002 "Evolution and speciation". Pp 225–250. *In*: Abatzopoulos, T. J., Beardmore, J. A., Clegg, J. S. & Sorgeloos, P. (Eds.). ***Artemia* Basic and Applied Biology**. Dor- drecht: Kluwer Academic Publishers.

Gajardo, G. M. & Beardmore, J. A. 2012. The brine shrimp *Artemia*: adapted to critical life conditions. **Frontiers in Physiology**, 3 Art. 185.

Gonçalves, R. J., Souza, M. S., Aigo, J., Modenutti, B., Balseiro, E., Villafañe, V. E., Cussac, V. & Helbling, E. W. 2010. Responses of plankton

and fish from temperate zones to UVR and temperature in a context of global change. **Ecología Austral**, 20: 129-153.

Gonçalves, R. J., Villafañe, V. E. & Helbling, E. W. 2002. Photorepair activity and protective compounds in two freshwater zooplankton species (*Daphnia menucoensis* and *Metacyclops mendocinus*) from Patagonia, Argentina. **Photochemistry and Photobiology Sciences**, 1: 996-1000.

Guermazi, W., Elloumi, J., Ayadi, H., Bouaïn, A. & Aleya, L. 2008. Coupling changes in fatty acid and protein composition of *Artemia salina* with environmental factors in the Sfax solar saltern (Tunisia). **Aquatic Living Resources**, 21: 63-73.

Guillard, R. R. L. & Ryther, J. H. 1962. Studies on marine planktonic diatoms *I. Cyclotella nana* Hustedt and *Detonula confervacae* (Cleve). **Canadian Journal of Microbiology**, 8: 229-239.

Ha, S. Y., Kim, Y. N., Park, M. O., Kang, S. H., Kim, H. & Shin, K. H. 2012. Production of mycosporine-like amino acids of in situ phytoplankton community in Kongsfjorden, Svalbard, Arctic. **Photochemistry and Photobiology**, 114: 1–14.

Häder, D-P, Helbling, E. W., Williamson, C. E. & Worrest, R. C. 2011. Effects of UV radiation on aquatic ecosystems and interactions with climate change. **Photochemistry and Photobiology Science**, 10 (2): 242-260.

Helbling, E. W., Menchi, C. F. & Villafañe, V. E. 2002. Bioaccumulation and role of UV-absorbing compounds in two marine crustacean species from Patagonia, Argentina. **Photochemistry and Photobiology Science**, 1: 820-825.

Hernández-Moresino, R. D., Gonçalves, R. J. & Helbling, E. W. 2014. Direct and indirect acquisition of photoprotective compounds in crab larvae of coastal Patagonia (Argentina). **Journal of Plankton Research**, 36 (3): 877-882.

Hernández-Moresino, R. D. & Helbling, E. W., 2010. Combined effects of UVR and temperature on the survival of crab larvae (Zoea I) from Patagonia: the role of UV-absorbing compounds. **Marine Drugs**, 8: 1681–1698.

Isiordia-Pérez, E., Puello-Cruz, A. C. & Gómez-Noguera, S. 2006. Efecto de diferentes dietas de microalgas sobre la supervivencia y crecimiento de *Apocyclopsaff panamensis* (Marsh 1913) (Copépodo: Cyclopoida) cultivado bajo condiciones controladas de laboratorio. **Revista Electronica de Veterinaria**, **REDVET**, 7: 1-9.

Johnson, M. W. & Olson, J. B. 1948. The life history and biology of a marine harpacticoid copepod, *Tisbe furcata* (Baird). **Biology Bulletin**, 95: 320-332.

Khairy, H. M. & El-Sayed, H. S. 2012. Effect of enriched *Brachionus plicatilis* and *Artemia salina* nauplii by microalga *Tetraselmis chuii* (Bütcher) grown on four different culture media on the growth and survival of *Sparus aurata* larvae. **African Journal of Biotechnology**, 11(2): 399–415.

Kicklighter, C. E., Kamio, M., Nguyen, L., Germann, M. W. & Derby, C. D. 2011. Mycosporine-like amino acids are multifunctional molecules in sea hares and their marine community. **Proceedings of the National Academy of Sciences of the United States of America**, 108: 11494–11499.

Korbee Peinado, N., Abdala Díaz, R. T., Figueroa, F. L. & Helbling, E. W. 2004. Ammonium and UV radiation stimulate the accumulation of mycosporine-like amino acids in *Porphyra columbina* (Rhodophyta) from Patagonia, Argentina. **Journal of Phycology**, 40: 248–259.

Lavens, P. & Sorgeloos, P. 2000. The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. **Aquaculture.** 181, 397–403.

Liang, Y., Beardall, J. & Heraud, P. 2006. Effects of nitrogen source and UV radiation on the growth, chlorophyll fluorescence and fatty acid composition of *Phaeodactylum tricornutum* and *Chaetoceros muelleri* (Bacillariophyceae). **Journal of Photochemistry and Photobiology B: Biology**, 82(3): 161-172.

Mallo, J. C., Fenucci, J. L. 2004. Feeding of protozoeal stages of the shrimp *Pleoticus muelleri*, Bate, with different microencapsulated food and microalgae species. **Revista de Biología Marina y Oceanografía,** 39: 13-19.

Mallo, J. C., Fenucci, J. L. & Galarza, C. M. 1999. Cría masiva de larvas y postlarvas del langostino argentino *Pleoticus muelleri* Bate (Crustacea, Decapoda, Solenoceridae). Pp.

313-317. *In*: Cabrera, T., Jory, D. & Silva, M. (Eds.). **Memorias Acuicultura** '99. World Aquaculture Society, Puerto La Cruz.

Marcoval, M. A., Villafañe, V. E. & Helbling, E. W. 2007. Interactive effects of ultraviolet radiation and nutrient addition on growth and photosynthesis performance of four species of marine phytoplankton. **Journal of Photochemistry and Photobiology B: Biology**, 89:78-87.

Mc Clinctock, J. B. & Karentz, D. 1997. Mycosporine-like aminoacids in 38 species of subtidal marine organisms from Mc Murdo Sound, Antarctica. **Antarctic Science**, 9: 392-398.

Mc Kenzie, R. L., Aucamp, P. J., Bais, A. F., Björn, L. O., Ilyas, M. & Madronich, S. 2010. Ozone depletion and climate change: impacts on UV radiation. Pp. 1-29. *In*: **Effects of solar UV radiation and climate change on biogeochemical cycling.** UNEP, EEAP-Report.

Moeller, R. E., Gilroy, S. H., 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.

Nahon, S., Charles, F., Lantoine, F., Vétion, G., Escoubeyrou, K., Desmalades & M., Pruski, A. M. 2010. Ultraviolet radiation negatively affects growth and food quality of the pelagic diatom *Skeletonema costatum*. **Journal of Experimental Marine Biology and Ecology**, 383: 164-170.

Neale, P. J., Banaszak, A. T. & Jarriel, C. R. 1998. Ultraviolet sunscreens in *Gymnodinium sanguineum* (Dinophyceae); mycosporine-like amino acids protect against inhibition of photosynthesis. **Journal of Phycology**, 34: 928-938.

Newman, S. J., Dunlap, W. C., Nicol, S. & Ritz, D. 2000. Antarctic krill (*Euphausia superba*) acquire UV-absorbing mycosporine- like amino acids from dietary algae. **Journal of Experimental Marine Biology and Ecology**, 255: 93–110.

Ogello, E. O., Nyonje, B. M. & Van Stappen, G. 2014. Genetic differentiation of *Artemia franciscana* (Kellogg, 1906) in Kenyan coastal saltworks. **International Journal of Advanced Research**, 2 (4): 1154-1164.

Oren, A. & Gunde-Cimerman, N. 2007. Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? **FEMS Microbiology Letters**, 269: 1–10.

Przeslawski, R. 2005. Combined effects of solar radiation and desiccation on the mortality and development of encapsulated embryos of rocky shore gastropods. **Marine Ecology Progress Series**, 298: 169-177.

Rastogi, R. P., Sinha, R. P., Singh, S. P., Häder, D-P. 2010 Photoprotective compounds from marine organisms. **Journal of Industrial Microbiology & Biotechnology**, 37: 537–558.

Riemer, U., Lamare, M. D. & Peake, B. M. 2007 Temporal concentrations of sunscreen compounds (Mycosporine-like Amino Acids) in phytoplankton and in the New Zealand krill, *Nyctiphanes australis* G.O. Sars. **Journal of Plankton Research,** 29: 1077–1086.

Shick, J. M. & Dunlap, W. C. 2002. Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. **Annual Review of Physiology**, 64: 223–262.

Støttrup, J. G., Bell, J. G. & Sargent, J. R. 1999. The fate of lipids during development and cold-storage of eggs in the calanoid copepod *Acartia tonsa* Dana, and in response to different algal diets. **Aquaculture**, 176: 257-269.

Tartarotti, B. & Torres, J. J. 2009. Sublethal stress: impact of solar UV radiation on protein synthesis in the copepod *Acartia tonsa*. **Journal of Experimental Marine Biology and Ecology**, 375: 106–113.

Thakur, D. P. & Lin, C. K. 2003. Water quality and nutrient budge in closed shrimp (*Penaeus monodon*) culture systems. **Aquaculture Engineering**, 27: 159–176.

Villafañe, V. E., Sundback, K., Figueroa, F. L. & Helbling, E. W. 2003. Photosynthesis in the aquatic environment as affected by UVR. Pp. 357–397. *In*: Helbling, E. W. & Zagarese, H. (Eds.). **UV Effects in Aquatic Organisms and Ecosystems**. Royal Society of Chemistry, Cambridge, UK.

Whitehead, K., Karentz, D. & Hedges, J. I. 2001. Mycosporine-like amino acids (MAAs) in phytoplankton, a herbivorous pteropod (*Limacina helicina*), and its pteropod predator

(*Clione antarctica*) in McMurdo Bay, Antarctica. **Marine Biology**, 139: 1013-1019.

Whitehead, K. & Vernet, M. 2000. Influence of mycosporine-like amino acids (MAAs) on UV absorption by particulate and dissolved organic matter in La Jolla Bay. **Limnology and Oceanography,** 45: 1788-1796.

Yoshiki, M., Tsuge, K., Tsuruta, Y., Yoshimura, T., Koganemaru, K., Sumi, T., Matsui, T. & Matsumoto, K. 2009. Production of new antioxidant compound from mycosporine-like amino acid, porphyra- 334 by heat treatment. **Food Chemistry**, 113: 1127–1132.

Zar, J. H. 1999. Biostatistical analysis, **Fourth ed. Prentice Hall**, Upper Saddle River, N.J. 929 p.

Zengling, M. A., Wei, L. I., Kunshan, G. A. O., 2012. Impacts of solar UV radiation on grazing, lipids oxidation and survival of *Acartia pacifica* Steuer (Copepod). **Acta Oceanologica**, Sin.31: 126–134.

Zudaire, L. & Roy, S. 2001. Photoprotection and long-term acclimation to UV radiation in the marine diatom *Thalassiosira weissflogii*. **Journal of Photochemistry and Photobiology B: Biology**, 62: 26-34.

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