ORIGINAL RESEARCH

Effects of dietary canthaxanthin on ultraviolet radiation stress in prawn *Artemesia longinaris*

Natalia S. Arzoz[1, 2, *], M. Alejandra Marcoval[1, 2], A. Cristina Díaz[1, 2, 3], M. Laura Espino[2, 3], Susana M. Velurtas[1, 2] and Jorge L. Fenucci[1, 2]

[1]Instituto de Investigaciones Marinas y Costeras (IIMyC), Universidad Nacional de Mar del Plata (UNMDP), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Juan B. Justo 2550 - Mar del Plata, Argentina. [2]Departamento de Ciencias Marinas, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (UNMdP), Funes 3350, B7602AYL - Mar del Plata Argentina. [3]Comisión de Investigaciones Científicas, Argentina. ORCID *Natalia S. Arzoz* https://orcid.org/0000-0002-9034-1719

ABSTRACT. The aims of this research were to investigate the effects of diets with added synthetic canthaxanthin (10% parafarm) and to evaluate its possible protective role under ultraviolet radiation (UVR) in prawn *Artemesia longinaris*. Three isoproteic and isolipidic diets (41% protein and 12% lipid) containing 0 (C_0), 100 (C_{100}), and 300 (C_{300}) mg of canthaxanthin kg^{-1} of diet were prepared. Before initiating the radiation experiment, prawns were fed with the different diets for a period of 21 d in order to determine a possible accumulation of carotenoids. Afterwards, animals were exposed to two radiation treatments for 7 d: a) photosynthetically active radiation (PAR, 400-700 nm), and b) total radiation (PAR+UVR, 280-700 nm), under controlled conditions (19 ± 2 °C, salinity = 33, pH = 7). In animals exposed to PAR+UVR treatment, survival varied between 50 and 83.33% with the highest value in animals fed diet C_{300}. At the end of the experiment, significant statistical differences were registered in integument carotenoid concentration. Under UVR stress, the highest decrease in non-polar carotenoid and esterified astaxanthin were recorded in prawns fed diets containing canthaxanthin. Scavenging properties were evaluated by electron resonance spectroscopy (EPR) using the stable 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical. Prawns fed with C_{300} showed the greatest activity to quench DPPH. Results suggested that dietary canthaxanthin could be acting as an antioxidant against reactive oxygen species and produced high tolerance under UVR stress.

Key words: Crustaceans, carotenoids, photoprotection, antioxidant activity.

*Correspondence:
natiarzoz@gmail.com

Received: 30 January 2023
Accepted: 10 May 2023

ISSN 2683-7595 (print)
ISSN 2683-7951 (online)

https://ojs.inidep.edu.ar

Journal of the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP)

Efectos de cantaxantina dietaria sobre el estres por radiacion ultravioleta en el camarón *Artemesia longinaris*

RESUMEN. Los objetivos de esta investigación fueron investigar los efectos de dietas adicionadas con cataxantina sintética (10% parafarm) y evaluar su posible papel protector bajo la radiación ultravioleta (RUV) en el camarón *Artemesia longinaris*. Se prepararon tres dietas isoprotéicas e isolipídicas (41% proteína y 12% lípidos) con 0 (C_0), 100 (C_{100}) y 300 (C_{300}) mg de cantaxantina kg^{-1} de dieta. Previo al experimento de radiación, los camarones fueron alimentados con las diferentes dietas durante 21 d para determinar una posible acumulación de carotenoides. Posteriormente, los animales fueron expuestos a dos tratamientos de radiación durante 7 d: a) radiación fotosintéticamente activa (PAR, 400-700 nm), y b) radiación total (PAR+RUV, 280-700 nm), bajo condiciones controladas (19 ± 2 °C, salinidad = 33, pH = 7). En los individuos expuestos al tratamiento PAR+RUV, la supervivencia varió entre 50 y 83,33%, con el valor más alto en animales alimentados con dieta C_{300}. Al final del experimento, se registraron diferencias estadísticas significativas en la concentración de carotenoides en el tegumento. Bajo estrés por RUV se registró la mayor disminución

de carotenoides no polares y astaxantina esterificada en camarones alimentados con dietas con cataxantina. La capacidad antioxidante se evaluó mediante espectroscopía de resonancia electrónica (EPR) utilizando el radical estable 2,2-difenil-2-picrilhidrazilo (DPPH). Los camarones alimentados con C_{300} mostraron la mayor actividad evidenciada por el decaimiento de DPPH. Los resultados sugirieron que la cantaxantina dietaria podría estar actuando como un antioxidante contra las especies reactivas de oxígeno y producir una alta tolerancia bajo estrés por RUV.

Palabras clave: Crustáceos, carotenoides, fotoprotección, actividad antioxidante.

INTRODUCTION

Carotenoids are natural pigments widely distributed in nature and synthesized *de novo* only by plants and some microorganisms, whereas animals depend on carotenoids supplementation from an exogenous diet to meet their metabolic nutritional requirements. In aquaculture, studies on carotenoid nutrition were initially focused on optimizing pigmentation levels (Niu et al. 2012; Wade et al. 2015). Subsequently, after recording the accumulation of carotenoids in tissues and organs across different species, further studies were conducted to explore other functions related to the health of cultured animals, with an emphasis on their role as antioxidants and their effects on growth and reproduction (Fenucci et al. 2015; Pereira da Costa y Miranda-Filho 2020).

Astaxanthin, which is the primary pigment in both the exoskeleton (95%) and muscle, has been evaluated as a dietary supplement (Carreto and Carignan 1984). Within the epidermal tissue, astaxanthin is mainly in the monoesterified form, while in the exoskeleton it is associated with proteins forming complexes called carotenoproteins (Wade et al. 2017). At present, alternative forms to increase the number of carotenoids in aquatic animal production are being studied. One of them is the addition of astaxanthin precursor carotenoids that crustaceans can metabolize and meet their needs (Pereira da Costa and Miranda-Filho 2020). In recent years, global warming affected the ozone layer increasing solar ultraviolet radiation (UVR 280-400 nm) on the Earth's surface (Barnes et al. 2019). Different species have developed mechanisms to minimize harmful effects of UVR, such as avoiding exposure through migrations in the water column, but not all organisms are mobile. For these organisms, defense consists of developing protection mechanisms (Roy 2000), being the production of structures to prevent penetration or the production of photoprotective compounds (PPCs) one of the most effective mechanisms (Marcoval et al. 2020; Arzoz et al. 2022).

The most common PPCs are UV-absorbing compounds (UACs) such as mycosporine-like amino acids (MAAs), which act as photoprotective UV filters and present a maximum absorption peak between 310 and 360 nm (Carreto and Carignan 1984) and carotenoids, which absorb within the range of 400-700 nm of visible radiation (Edge 1997) and have the ability to inactivate molecules in an electronically excited state, mainly those due to photosensitive reactions. Among defense strategies, carotenoids are most likely involved in avoiding the photooxidative damage initiated by reactive oxygen species (Stahl and Sies 2003). The presence of free or esterified forms of various carotenoids has been reported in different tissues in wild and cultured crustaceans (Lenel et al. 1978; Castillo et al. 1982). Among those that have been isolated from various classes of crustaceans are non-polar carotenoids, like β-carotene, and polar carotenoids, like astaxanthin, equinenone, and canthaxanthin. Over the last decades, along with the rapid growth of aquaculture and the development of new techniques, stressors have also emerged harming the health of farmed animals and generating economic losses (FAO 2020).

The evaluation of biological functions of different compounds/additives, such as amino acids, essential fatty acids, phospholipids, vitamins, minerals, carotenoids, different synthetic chemical products and derivatives of bacteria, yeasts, fungi and algae, plants and animals (Ciji and Akhtar 2021) for stress migration, arises in this context.

Studies to evaluate whether dietary carotenoids, mainly astaxanthin, could improve stress tolerance to different factors such as temperature, salinity (Chen et al. 2018), nitrite (Díaz et al. 2014) and ammonia (Pan et al. 2003) in aquatic animals have been designed. However, there is no information available on the possible photoprotective role under stress by ultraviolet radiation (UVR), which is regaining importance for the cultivation of organisms such as peneids. This research focused on juvenile prawn *Artemesia longinaris*, an exclusive species found in the South American Atlantic and distributed along the coast from 23° S to 43° S. This species is an important fishing resource of high commercial value in different countries (Bauer 2020). Several aspects of the growth and nutrition of this species have been studied (Gimenez et al. 2002; Diaz et al. 2017; Arzoz et al. 2022). However, the possible photoprotective role of synthetic canthaxanthin has not yet been evaluated and in the current context of climate change, this is a relevant aspect for its cultivation, which is carried out in shallow ponds where UVR penetrates to the bottom of the water column (Villafañe et al. 2003).

The aims of this research were to determine the bioaccumulation of carotenoids from canthaxanthin-added diets in juveniles of *A. longinaris* and to evaluate their possible protective effects under ultraviolet radiation (UVR) stress conditions. For this purpose, the effects of the interaction between canthaxanthin concentration in the feed and the exposure to UVR were evaluated in terms of survival, carotenoid accumulation in the integument, and free radical scavenging.

MATERIALS AND METHODS

Experimental animals

Juveniles of *A. longinaris* collected with a trawling net from Mar del Plata coastal waters (38° 02′ S, 57° 30′ W) were transferred to J. J. Nagera Coastal Station, National University of Mar del Plata (38° 16′ S, 63° 30′ W). They were kept for one week in 3,500-l cylindrical fiberglass tanks for acclimatization to laboratory conditions (18 ± 1 °C, pH = 7 and salinity = 33). Individuals were starved for the first 24 h to empty their gut contents and were then fed the control diet (C_0) once a day during the 10-day acclimatization period (Table 1).

Before the radiation experiment, to determine a possible accumulation of carotenoids, prawns (3.09 ± 0.58 g; n = 90) were placed in individual 20-l glass aquaria connected to a recirculating system having a packed-column biological filter for the removal of nitrogenous wastes, under controlled conditions (19 ± 2 °C, salinity = 33, pH = 7). Different dietary treatments were assigned to 30 aquaria, each aquarium containing 1 prawn (total = 90; 30 replicates per diet). Specimens were fed three isoproteic and isolipidic diets (41% protein and 12% lipid) for a 21-day period supplemented with 0 (diet C_0), 100 (diet C_{100}), and 300 (diet C_{300}) mg of synthetic canthaxanthin kg^{-1} of diet (Table 1). All ingredients were mixed and pelletized using the cold extrusion method (Diaz and Fenucci 2002) and dried for 24 h at 50 °C.

At the end of the 21-day period of carotenoid accumulation, prawns were exposed during 7 d to two radiation treatments: photosynthetically active radiation (PAR, range 400-700 nm) and total radiation spectrum (PAR+UVR, range 280-700 nm) while they continued to be fed with their respective diets. To test each treatment, six 20-l aquaria with two animals each were exposed to two radiation and two feeding treatments with the

Table 1. Composition of different diets. C_0: control diet, C_{100}: diet added with 100 mg of canthaxanthin kg^{-1} diet, C_{300}: diet added with 300 mg of canthaxanthin kg^{-1} diet.

Ingredient (g 100 g^{-1})	C_0	C_{100}	C_{300}
Fish meal (65% protein)[a]	48	48	48
Soybean meal (42% protein)[b]	17	17	17
Corn starch	20	20	20
Squid protein (85% protein)	1	1	1
Wheat bran	8.5	8.49	8.47
Canthaxanthin[c]	0	0.01	0.03
Fish oil	2	2	2
Fish soluble	2	2	2
Soybean lecithin	0.5	0.5	0.5
Cholesterol	0.5	0.5	0.5
Vitamins[d]	0.5	0.5	0.5

[a]Agustinier S.A., Mar del Plata, Argentina.
[b]Melrico S.A., Argentina.
[c]Synthetic Canthaxanthin for veterinary use 10% parafarm: dark brown granulated powder with violet reflections. Rating (11.31%, on dry basis).
[d]g kg^{-1}: cholecalciferol 1.8, thiamin 8.2, riboflavin 7.8, pyridoxine 10.7, calcium panthothenate 12.5, biotin 12.5, niacin 25.0, folic acid 1.3, B12HCl 1.0, ascorbic acid (Rovimix Stay C) 39.1, menadione 1.7, inositol 0.3, cholinechloride 0.2, a-tocopherolacetate 75, vitamin A acetate 5.0.

follow design: Treatment A: PAR-C_0 (control treatment); Treatment B: PAR-C_{100}; Treatment C: PAR-C_{300}; Treatment D: PAR+UVR-C_0; Treatment E: PAR+UVR-C_{100}; and Treatment F: PAR+UVR-C_{300}. Light sources were 40 W cool-white fluorescent bulbs (Philips) for photosynthetically active radiation (PAR) and Q-Panel UV-A-340 bulbs for UVR. Average irradiances were 65.4 W m^{-2} for PAR and 20 W m^{-2} for UVR (Marcoval et al. 2021), and intensities were set according to Arzoz et al. (2022). At the end of the exposure period, survival of different aquaria was estimated and samples of integument and midgut gland were taken.

Carotenoid analysis

After exposure, animals were cryoanesthezied to take integument samples. Dissected parts were freeze-dried and subsequently homogenized in an argon atmosphere in darkness. Carotenoids were analyzed following Schiedt (1993) modified by Díaz et al. (2013). Non-polar carotenoids were extracted three times with hexane in an argon atmosphere in darkness. Free astaxanthin was separated by partitioning with dimethyl sulphoxide (DMSO)/acetone (1:3) until colorless in an inert atmosphere. Esterified astaxanthin was extracted according to Napoli and Horst (1989). Concentrations in µg g^{-1} tissue were calculated using standard curves of β-carotene in hexane (1.88×10^{-6} M), astaxanthin in DMSO/acetone (4.19×10^{-6} M), and extinction coefficients of β-carotene 122,000 and astaxanthin 124,000 (Perkampus 1992).

Antioxidant activity

Potential antioxidant activity in midgut gland homogenates was determined based on the scav-

enging activity of the stable 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical. Lyophilized midgut gland (20 mg) from each treatment was mixed with 1 ml of chloroform under an argon atmosphere and different reaction mixtures contained DPPH (6.67×10^{-6} M) were prepared according to Diaz et al. (2014), and the radical scavenging activity was determined using paramagnetic electronic resonance according to Arzoz et al. (2022).

Statistical analysis

Carotenoid concentrations were compared by means analysis of variance (one-way ANOVA). In addition, to compare and determine the degree of significance of survival results, the arcsine transformation was applied to the percentages and one-way ANOVA was performed. Results obtained in the tests of total antioxidant activity were estimated by an analysis of covariance (ANCOVA). Data were reported as mean ± standard deviation. Analyses were performed with Vassart stats, with a significance level of $\alpha = 0.05$.

RESULTS

Survival percentages after a week of exposure to different radiation and feeding treatments varied between 100 and 83.33% in prawns kept under PAR treatment, and between 50 and 83.33% for animals exposed to UVR treatment. The highest value was observed in animals fed diet C_{300}, and a significant decrease (ANOVA $p = 0.01$) was registered in animals fed diets C_0 (treatment D) and C_{100} (treatment E) (Figure 1).

After 28 days of feeding, significant statistical differences were registered in carotenoid concentration between treatments (Table 2). Significant increases in non-polar carotenoid concentration (ANOVA $p = 0.01$ and $p = 0.002$, respectively) and free astaxanthin (ANOVA $p = 0.0008$ and $p = 0.03$, respectively) were observed in animals under PAR treatments (B and C), compared to those fed diet C_0 (treatment A). In treatments under UVR, animals fed diets C_{100} and C_{300} (treatments E and F) showed a statistically signif-

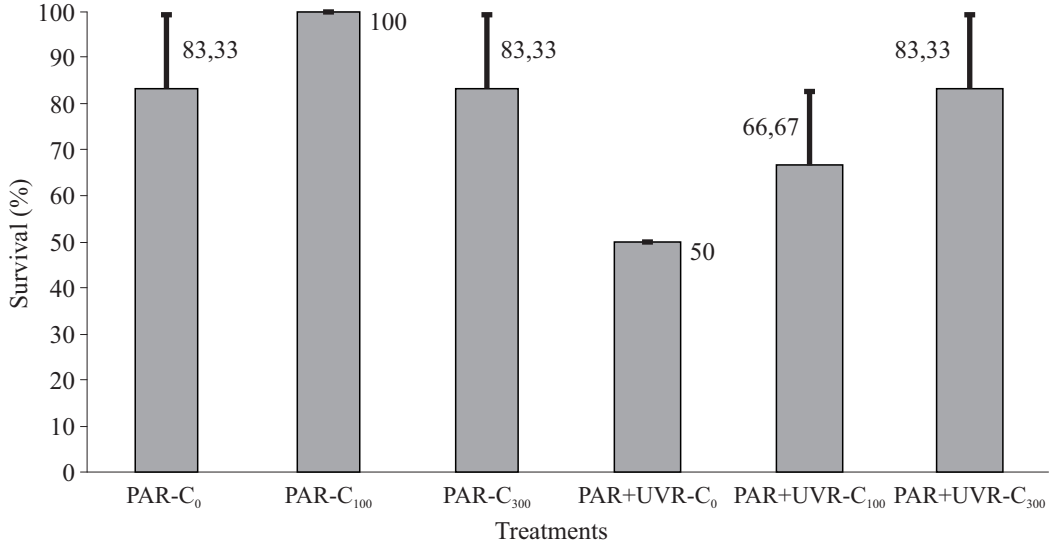

Figure 1. Survival percentages after a week of exposure to different radiation and feeding treatments. PAR: photosynthetic active radiation, PAR+UVR: PAR + ultraviolet radiation, C_0: control diet, C_{100}, and C_{300}: control diet supplemented with 100 and 300 mg of canthaxanthin kg^{-1} diet. Values are expressed as mean ± SE, n = 6.

Table 2. Carotenoids concentration (μg g^{-1} tissue) of juveniles *A. longinaris* fed diets added with different concentrations of canthaxanthin and exposed to different radiation treatments for a period of 21 d. PAR: photosynthetic active radiation, UVR: ultraviolet radiation, C_0: control diet, C_{100}, C_{300}: control diet supplemented with 100 and 300 mg of canthaxanthin kg^{-1} of diet (n = 3), respectively. Means in a row with different superscripts indicate significant differences (ANOVA $p < 0.05$).

	PAR			PAR+UVR		
	A-diet C_0	B-diet C_{100}	C-diet C_{300}	D-diet C_0	E-diet C_{100}	F-diet C_{300}
Non-polar carotenoids	2.86 ± 0.92^a	5.51 ± 1.01^b	5.63 ± 0.57^b	2.83 ± 0.60^a	1.44 ± 0.12^c	1.07 ± 0.38^c
Free astaxanthin	1.28 ± 0.43^d	2.73 ± 0.51^e	2.78 ± 0.88^e	1.40 ± 0.93^d	0.78 ± 0.11^d	0.77 ± 0.17^d
Esterified astaxanthin	1.04 ± 0.26^f	1.68 ± 0.49^f	1.22 ± 0.49^f	1.42 ± 0.43^f	0.58 ± 0.12^g	0.67 ± 0.25^g
Total	5.18	9.92	9.63	5.65	2.8	2.51

icant decrease in non-polar carotenoid (ANOVA $p = 0.02$ and $p = 0.01$, respectively) and esterified astaxanthin ($p = 0.02$ and $p = 0.03$, respectively) compared to those of treatment D.

In all treatments, midgut gland extract had antioxidant protective capacity evidenced by the ability to react with the DPPH radical (Figure 2). In the first 3 min of reaction, the percentage of remaining DPPH was similar for all treatments (between 82 and 95%) except for prawns fed C_{300} and exposed to UVR (treatment F), in which the signal decayed drastically (60%) (ANCOVA $p = 0.025$). Statistically significant differences (ANCOVA $p = 0.020$) were registered between light treatments only in prawns fed diet C_{300}, with 82% and 60% of the remaining DPPH percentage for PAR and PAR+UVR treatments, respectively.

DISCUSSION

In aquaculture, carotenoids have been used mainly as a source of pigment; however, potential functions as feed additives have been inferred from them. Several studies have demonstrated the critical role of carotenoids in increasing stress tolerance in aquatic animals (Lim et al. 2023). While carotenoids from both natural and synthetic sources have been evaluated, astaxanthin has received considerable attention due to its popularity as one of the most effective naturally occurring antioxidants (Lim et al. 2018).

The present study represents the first assessment of the effects of diets added with different synthetic canthaxanthin concentrations on prawn *A. longinaris* under UVR stress. Among peneid, most of the studies have been carried out in shrimp *Penaeus monodon* and *Litopenaeus vannamei* (Wade et al. 2017). It has been demonstrated that astaxanthin improved tolerance to thermal, osmotic (Chien et al. 2003) and ammonia stress (Pan et al. 2003) in *P. monodon.* On the other hand, Quintana López et al. (2019) observed in the white shrimp *P. vannamei* that the accumulation of astaxanthin depended on the shrimp rearing system, with the content of esterified astaxanthin being higher in the midgut gland, exoskeleton, and muscles of shrimps reared under extensive conditions compared to those reared under hyperintensive conditions. These results suggest that astaxanthin could be helpful during stress conditions in shrimp farming. Recently, Zhao et al. (2022) showed that astaxanthin also improved immunity and alleviated oxidative and ammonia stress. Canthaxanthin is one of the intermediates

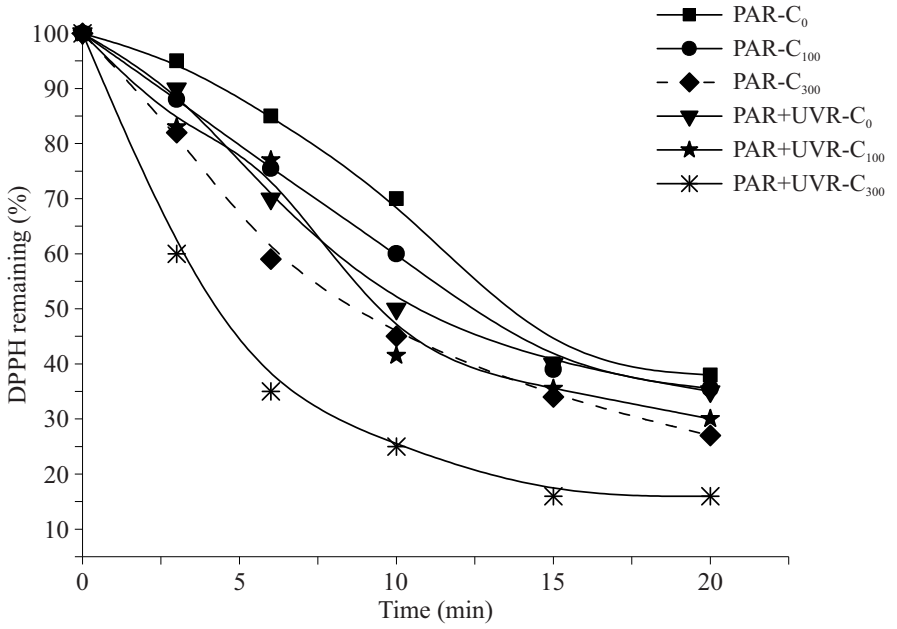

Figure 2. Free-radical 2,2-diphenyl-2-picrylhydrazyl (DPPH) reaction kinetics of the midgut gland of juveniles of *A. longinaris* fed diets C_0, C_{100} and C_{300} exposed to various light treatments over a period of 7 d. All measurements were made in triplicate and mean values were plotted. C_0: control diet; C_{100}, C_{300}: control diet supplemented with mg of canthaxanthin kg^{-1} diet.

in the metabolic pathway of carotenoids, and shrimps can convert synthetic canthaxanthin from feed and deposit it in their tissues as astaxanthin (Boonyaratpalin et al. 2001). Niu et al. (2012) registered improvement in survival against low DO stress in *P. monodon* fed with dietary canthaxanthin. Fawzy et al. (2022) evaluated the use of diets supplemented with 0 (control), 50, 100, 200, and 400 mg of canthaxanthin kg^{-1} of diet in *L. vannamei* and demonstrated that supplementation in the range of 173.73 to 202.13 mg is of primary importance for shrimp growth and health status. Similarly, in the present work, survival was higher in animals fed diets added with canthaxanthin (66.67% and 83.33%) under UVR stress. The same percentage (83.33%) was recorded in both treatment PAR and PAR+UVR in animals fed diet C_{300}. These results suggest that the inclusion of canthaxanthin (precursor of astaxanthin) with lower commercial cost, improved survival in *A. longinaris* juveniles under UVR stress.

Quantitative and qualitative distribution of carotenoids in aquatic animals is mainly the result of species-specific dietary habits, metabolic absorption, and transformation capacity of different carotenoids. The type of pigments absorbed and the specific absorption rates can vary considerably between families or species (Meyers 2000). In the present study, animals under PAR treatments and fed different diets exhibited significant differences in carotenoid concentrations. In those specimens fed diets added with canthaxanthin, concentrations of non-polar carotenoids and free astaxanthin were higher than in animals fed the basal diet. Results obtained for non-polar carotenoids coincide with previous studies in which an increase in non-polar carotenoids, such as β-carotene, was recorded in shrimp fed diets added with carotenoids (Díaz et al. 2011; Pisani et al. 2014). This difference could be due to oxidative reactions of astaxanthin formation from non-polar carotenoids, such as β-carotene, which

require more energy than reactions from canthaxanthin (Ando et al. 1986). On the other hand, differences in free astaxanthin concentration could be due to the ability of these animals to synthesize astaxanthin from dietary canthaxanthin (Wade et al. 2017). Differences in carotenoid concentrations were also observed in animals under UVR treatment. A significant decrease in non-polar carotenoid concentration and esterified astaxanthin was recorded. These results are coincident with the determination of antioxidant activity which was higher ($p = 0.025$, $n = 3$) in animals fed diets C_{300} compared to those fed diets C_0 and $C_{100,}$ detecting a DPPH remnant of 60% after 3 min.

Carotenoids play an important role in animal health as an antioxidant through the inactivation of free radical produced by their own metabolism or by environmental factors (Kirti et al. 2014). There are several mechanisms of carotenoid action as an antioxidant, including: serving as efficient physical quenchers of excited singlet molecular oxygen ($1O_2$) (Widomska et al. 2019; Kruk et al. 2021) reacting rapidly with free radicals of different origins and convert them into more stable compounds or non-radical products (Focsan et al. 2017, 2021); preventing the formation of free radicals through the interruption of free radical-induced chain reactions and terminating free radical oxidations (Lai et al. 2020); and acting as metalchelators by facilitating the conversion of iron and copper derivatives into stable chelate complexes (Focsan et al. 2017). Díaz et al. (2013) demonstrated that tissues of *P. muelleri* post-larvae registered (with higher carotenoid concentrations) a higher percentage of DPPH decay over time, probably because radicals are consumed in the tissue at a rate that depends on concentrations of protective substances. Díaz et al. (2014) registered an increase in antioxidant capacity in post-larvae of this species fed a diet supplemented with 100 and 300 mg of astaxanthin kg^{-1} of diet and exposed to nitrite stress. In agreement with the present study, *A. longinaris* under stress by UVR generated greater antioxidant capacity when fed with a diet added with 300 mg of canthaxanthin.

Results obtained in this study provide evidence that the addition of synthetic canthaxanthin to the diet of *A. longinaris* protects them against UVR stress under culture conditions and suggest that dietary canthaxanthin could be acting as an antioxidant against reactive oxygen species generated by exposure to UVR. For the culture of *A. longinaris*, the recommended concentration should be 300 mg kg^{-1} of diet, amount by which survival was not affected during exposure to UVR and antioxidant activity was significantly higher. These results are consistent with finding in several fish (Rahman et al. 2016; Yi et al. 2018; Bacchetta et al. 2019) and other crustaceans (Ettefaghdoost et al. 2021; Fawzy et al. 2022) in which dietary carotenoids improved their total antioxidant status while activities of antioxidant enzymes like catalase and superoxide dismutase decreased. Those authors proposed that carotenoids are comparatively more potent free radical quenchers than antioxidant enzymes and, as such, superseded their functional importance. Although it has been shown that carotenoid-fortified feeds stimulate the antioxidative capacity and stress tolerance in penaeid shrimp, more comprehensive and rigorous research is still necessary to establish the explicit functional association between antioxidant defense and carotenoid-modulated immune mechanisms and their positive contribution to aquatic animal health and stress relief.

Competing interests

The authors declare there are no competing interests.

Funding

This work was supported by the Consejo Nacional de Investigaciones Científicas y Técni-

cas (Grant Number 112201101-00019) and the Universidad Nacional de Mar del Plata, Argentina (Grant Number EXA15/E 827).

Data availability

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Ethics

Authors confirm that the present work was developed in compliance with the Ethical Principles for Research in Laboratory, Farm and Nature Laboratory Animals of the National Scientific and Technical Research Council (CONICET-OCR-RD-20050701-1047) of Argentina.

Author contributions

Natalia S. Arzoz: writing-original draft, writing-review and editing. M. Alejandra Marcoval investigation, conceptualization, supervision, writing-review and editing. A. Cristina Díaz: data curation, formal analysis, writing-review and editing. M. Laura Espino: software, writing-review and editing. Susana M. Velurtas: data curation, writing-review and editing. Jorge L. Fenucci: project administration, funding acquisition, writing-review and editing.

REFERENCES

Ando S, Takeyama T, Hatano M. 1986. Isolation and characterization of a carotenoid-carrying lipoprotein in the serum of Chum salmon (*Oncorhynchus keta*) during spawning migration. Agric Biol Chem. 50: 907-914.

Arzoz NS, Marcoval MA, Díaz AC, Espino ML, Velurtas MS, Churio MS, Orallo DE, Fenucci JL. 2022. Photoprotection effects of red seaweed meal (*Grateloupia* cf. *turuturu*) as feed ingredient in prawn *Artemesia longinaris.* Photochem Photobiol. 98 (6): 1476-1485.

Bacchetta C, Rossi AS, Cian RE, Drago SR, Cazenave J. 2019. Dietary β-carotene improves growth performance and antioxidant status of juvenile *Piaractus mesopotamicus*. Aquacult Nutr. 25 (4): 761-769. DOI: https://doi.org/10.1111/anu.12893

Barnes PW, Williamson CE, Lucas RM, Robinson SA, Madronich S, Nigel D, Bornman JF, Bais AF, Sulzberger B, Wilson SR, et al. 2019. Ozone depletion, ultraviolet radiation, climate change and prospects for a sustainable future. Nat Sust. [accessed 2021 Apr 25]; 2: 569-579. DOI: https://doi.org/10.1038/s41893-019-0314-2

Bauer RT. 2020. Shrimp fisheries. Fish Aquacult. 9: 91-116.

Boonyaratpalin M, Thongrod S, Supamattaya K, Britton G, Schlipalius LE. 2001. Effects of beta-carotene source, *Dunaliella salina*, and astaxanthin on pigmentation, growth, survival and health of *Penaeus monodon*. Aquac Res. 32: 182-190.

Carreto JI, Carignan MO. 1984. Pigmentos carotenoides del camarón *Artemesia longinaris* Bate (Crustacea, Decapoda, Penaeidae). Rev Invest Desarr Pesq. 4: 5-20.

Castillo R, Geneviève L, Roland L. 1982. General Survey of the carotenoids in Crustacea. In: Britton G, Goodwin T, editors. Carotenoid chemistry and biochemistry. Pergamon: Elsevier. p. 211-224.

Chen SJ, Guo YC, Espe M, Yang F, Fang WP, Wan MG, Tian LX. 2018. Growth performance, haematological parameters, antioxidant status and salinity stress tolerance of juvenile Pacific white shrimp (*Litopenaeus vannamei*) fed different levels of dietary myo-inositol. Aquac Nutr. 24 (5): 1527-1539.

Chien YH, Pan C-H, Hunter B. 2003. The resistance to physical stresses by *Penaeus monodon*

juveniles fed diets supplemented with astaxanthin. Aquaculture. 216: 177-191.

Ciji A, Akhtar MS. 2021. Stress management in aquaculture: a review of dietary interventions. Rev Aquacult. 13 (4): 2190-2247.

Díaz AC, Espino ML, Arzoz NS, Velurtas SM, Ponce, NMA, Stortz, CA, Fenucci JL. 2017. Free radical scavenging activity of extracts from seaweeds *Macrocystis pyrifera* and *Undaria pinnatifida*: applications as functional food in the diet of prawn *Artemesia longinaris*. Lat Am J Aquat Res. 45 (1): 104-112.

Díaz AC, Fenucci JL. 2002. Comparative evaluation of different animal protein source in juveniles of *Pleoticus muelleri* (Crustacea, Penaeoidea). In: Escobar-Briones E, Alvarez F, editors. Modern approaches to the study of Crustacea. New York: Kluwer Academy Plenum Publish. p. 75-78.

Díaz AC, Velurtas SM, Espino ML, Fenucci JL. 2014. Effect of dietary astaxanthin on free radical scavenging capacity and nitrite stress tolerance of postlarvae shrimp, *Pleoticus muelleri.* J Agr Food Chem. 62: 12326-12331.

Díaz AC, Velurtas SM, Fernandez Gimenez AV, Mendiara SN, Fenucci JL. 2011. Carotenoids from integument, muscle and midgut gland of the red shrimp *Pleoticus muelleri* (Bate, 1888) (Crustacea, Penaeoidea). Isr J Aquacult-Bamid. 63: 625-631.

Díaz AC, Velurtas SM, Mendiara SN, Fenucci JL. 2013. Correlation between radicals scavenging capacity and carotenoid profile during larval development of *Pleoticus muelleri*. Invertebr Reprod Dev. 51 (1): 43-48.

Edge R, McGarvey DJ, Truscott, TG. 1997. The carotenoids as anti-oxidants a review. J Photochem Photobio B. 41: 189-200.

Ettefaghdoost M, Haghighi H. 2021. Impact of different dietary lutein levels on growth performance, biochemical and immunophysiological parameters of oriental river prawn (*Macrobrachium nipponense*). Fish Shellfish Immunol. 115: 86-94. DOI: https://doi.org/10.1016/j.fsi.2021.05.024

[FAO] Food and Agriculture Organization of the United Nations. 2020. El estado mundial de la pesca y la acuicultura 2020. La sostenibilidad en acción. Roma: FAO. 243 p. DOI: https://doi.org/10.4060/ca9229es

Fawzy S, Wang W, Wu M, Yi G, Huang X. 2022. Effects of dietary different canthaxanthin levels on growth performance, antioxidant capacity, biochemical and immune-physiological parameters of white shrimp (*Litopenaeus vannamei*). Aquaculture. 556: 738276.

Fenucci JL, Pisani E, Díaz AC, Velurtas SM. 2015. Effect of dietary carotenoids on Argentine red shrimp broodstock. J World Aquacult Soc. 46 (1): 57-60.

Focsan AL, Kispert LD. 2017. Radicals formed from proton loss of carotenoid radical cations: a special form of carotenoid neutral radical occurring in photoprotection. J. Photochem Photobiol B Biol. 166: 148-157. DOI: https://doi.org/10.1016/j.jphotobiol.2016.11.015

Focsan AL, Polyakov NE, Kispert LD. 2021. Carotenoids: importance idaily life-insight gained from EPR and ENDOR. Appl Magn Reson. 52 (8): 1093-1112.

Gimenez AF, Garcia-Carreno FL, Del Toro MN, Fenucci, JL. 2002. Digestive proteinases of *Artemesia longinaris* (Decapoda, Penaeidae) and relationship with molting. Comp Biochem Physiol B Biochem Mol Biol.132 (3): 593-598.

Kirti K, Amita S, Priti S, Jyoti S. 2014. Colorful world of microbes: carotenoids and their applications. Adv Biol. 837891. 13 p.

Kruk J, Szymanska R. 2021. Singlet oxygen oxidation products of carotenoids, fatty acids and phenolic prenyl lipids. J Photochem Photobiol B Biol. 216: 112-148.

Lai TT, Yang CM, Yang CH. 2020. Astaxanthin protects retinal photoreceptor cells against high glucose-induced oxidative stress by induction of antioxidant enzymes via the PI3K/Akt/Nrf2 pathway. Antiox. 9 (8): 729.

DOI: https://doi.org/10.3390/antiox9080729

Lenel R, Negre-Sadargues G, Castillo R. 1978. Carotenoid pigments in crustacean. Archs Zool Exp Gen. 119: 297-334.

Lim KC, Yusoff FM, Karim M, Natrah FM. 2023. Carotenoids modulate stress tolerance and immune responses in aquatic animals. Rev Aquac. 15 (2): 872-894.

Lim KC, Yusoff FM, Shariff M, Kamarudin, MS. 2018. Astaxanthin as feed supplement in aquatic animals. Rev Aquac. 10 (3): 738-773.

Marcoval MA, Díaz AC, Espino ML, Arzoz NS, Velurtas MS, Fenucci JL. 2020. Role of dietary photoprotective compounds on the performance of shrimp *Pleoticus muelleri* under UVR stress. Aquaculture. 515: 734564. DOI: https://doi.org/10.1016/j.aquaculture.2019.734564

Marcoval MA, Pan J, Díaz AC, Fenucci JL. 2021. Dietary bioaccumulation of UV-absorbing compounds, and post-ingestive fitness in larval planktotrophic crustaceans from coastal SW Atlantic. Mar Environ Res. 170, 105433.

Meyers SP. 2000. Papel del carotenoide astaxantina en nutrición de especies acuáticas. In: Civera Cerecedo R, Pérez-Estrada CJ, Ricque-Marie D, Cruz-Suárez L, editors. Avances en Nutrición Acuícola IV. Memorias del IV Simposium Internacional de Nutrición Acuícola La Paz, BCS, México. p. 473-491.

Napoli JL, Horst RL. 1989. Quantitative analyses of naturally occurring retinoid. In: Redfern CP, editor. Retinoid protocols human. New Jersey: Press Totowa. p. 29-41.

Niu J, Li H, Liu Y, Tian J, Chen LX, Huang X, Lin YHZ. 2012. Dietary values of astaxanthin and canthaxanthin in *Penaeus monodon* in the presence and absence of cholesterol supplementation: effect on growth, nutrient digestibility and tissue carotenoid composition. Br J Nut. 8: 80-91.

Pan CH, Chien YH, Hunter B. 2003. The resistance to ammonia stress of *Penaeus monodon* Fabricius juvenile fed diets supplemented with astaxanthin. J Exp Mar Biol Ecol. 297 (1): 107-118.

Pereira da Costa D, Campos Miranda-Filho K. 2020. The use of carotenoid pigments as food additives for aquatic organisms and their functional roles. Rev Aquacult. 12: 1567-1578.

Perkampus HH. 1992. UV-VIS Atlas of organic compounds. Part 2. 2nd ed. New York: VCH Wiley. 1525 p.

Pisani E, Díaz AC, Velurtas SM, Fenucci JL. 2014. Effect of dietary carotenoids in the shrimp broodstock *Artemesia longinaris*. XV Jornadas anuales de la Sociedad Argentina de Biología. Biocell 38 (2).

Quintana-López A, Hurtado-Oliva MA, Manzano-Sarabia M, López-Peraza DJ, Hernández C, García A, Palacios E. 2019. Effect of rearing conditions on astaxanthin accumulation in the white shrimp *Penaeus vannamei* (Boone, 1931). Lat Am J Aquat Res. 47 (2): 303-309.

Rahman M, Khosravi S, Chang KH, Lee S. 2016. Effects of dietary inclusion of astaxanthin on growth, muscle pigmentation and antioxidant capacity of juvenile rainbow trout (*Oncorhynchus mykiss*). Prev Nutr Food Sci. 21 (3): 281-288. DOI: https://doi.org/10.3746/pnf.2016.21.3.281

Roy S. 2000. Strategies for the minimization of UV-induced damage. In: De Mora S., Demers S, Vernet M, editors. The effects of UV radiation in the marine environment. Cambridge: Cambridge University Press. p. 177- 205.

Schiedt K, Bischof S, Glinz E. 1993. Metabolism of carotenoids and *in vivo* racemization of astaxanthin in the crustacean *Penaeus*. Meth Enzymol. 214: 148-168.

Stahl W, Sies H. 2003. Antioxidant activity of carotenoids. Mol Aspects Med. 24: 345-351.

Villafañe VE, Sundback K, Figueroa FL, Helbling EW. 2003. Photosynthesis in the aquatic environment as affected by UVR. In: Helbling ER, Zagarese H, editors. UV effects in aquatic organisms and ecosystems. Cam-

bridge: Society of Chemistry. p. 357-397.

Wade NM, Budd A, Irvin S, Glencross BD. 2015. The combined effect of diet, environment and genetics on pigmentation in the giant tiger prawn, *Penaeus monodon*. Aquaculture. 449: 78-86. DOI: https://doi.org/10.1016/j.aquaculture.2015.01.023

Wade NM, Gabaudan J, Glencross BDA. 2017. Review of carotenoid utilization and function in crustacean aquaculture. Rev Aquacult. 9: 141-156.

Widomska J, Welc R, Gruszecki WI. 2019. The effect of carotenoids on the concentration of singlet oxygen in lipid membranes. BBA-Biomembranes. 1861 (4): 845-851. DOI: https://doi.org/10.1016/j.bbamem.2019.01.012

Yi X, Shen H, Li J, Wei Z, Shentu J, Zhang W, Mai K. 2018. Effects of dietary vitamin E and astaxanthin on growth, skin colour and antioxidative capacity of large yellow croaker *Larimichthys crocea*. Aquacult Nutr. 24 (1): 472-480. DOI: https://doi.org/10.1111/anu.12580

Zhao X, Wang G, Liu X, Guo D, Chen X, Liu S, Li G. 2022. Dietary supplementation of astaxanthin increased growth, coloration, the capacity of hypoxia and ammonia tolerance of Pacific white shrimp (*Litopenaeus vannamei*). Aquacult Rep. 23: 101093.