Effect of Dietary Carotenoids on Argentine Red Shrimp Broodstock

Jorge L. Fenucci, Emiliano Pisani, Ana Cristina Díaz and Susana M. Velurtas

The Argentine Red Shrimp

The Argentine red shrimp *Pleoticus muelleri* (Fig. 1) is an open-thelycum penaeid shrimp species that occurs in cold temperate waters along the South American coast from Rio de Janeiro, Brazil (22°S) to Santa Cruz, Argentina (50°S). The complete life cycle takes place in the sea, without the shrimp entering coastal estuaries, although a clear migration occurs between shallow waters and those of greater depth where spawning takes place. Adults occupy mid-water and benthic habitats, associated with mud and fine sand substrates in the subtidal zone, at depths ranging from 3 to 100 m. The dietary feeding approach of this species can be described as omnivorous.

The regions of greatest abundance occur at temperatures from 6 to 23 C and salinities between 31.5 and 33.5 g/L. According to the total declared catches for 2001-2011, fishery yields of this species were maximum in 2001 and 2011 at 80,000 t and minimum in 2005 at almost 7,000 t (Fig. 2). Males can reach 50 g and females 90 g. Spawning capacity in captivity can reach over 360,000 eggs per female (Díaz and Fenucci 2004).

The aquaculture group from the National University of Mar del Plata has been working on different aspects of the biology, nutrition, maturation, large-scale larval culture and grow-out of Argentine red shrimp. This species has garnered great interest because of its potential use in aquaculture in temperate areas.

FIGURE 1. *Female Argentine red shrimp* Pleoticus muelleri.

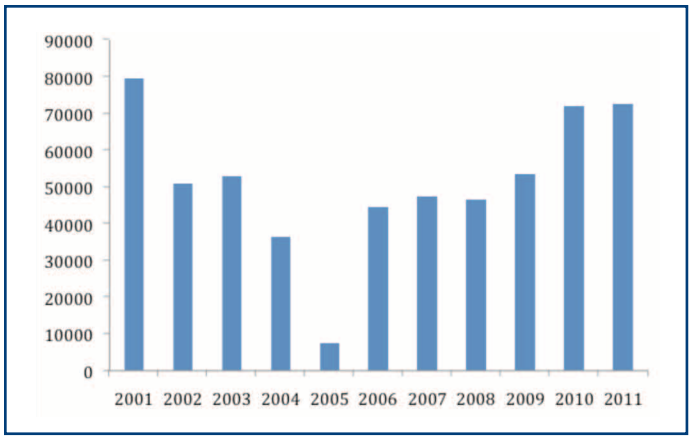

FIGURE 2. *Total catch (t) of Argentine red shrimp between 2001 and 2011.*

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Carotenes and Broodstock Nutrition

Broodstock nutrition is an important factor in the success or failure of reproduction. Feeding captive crustaceans is limited to artificial diets that may lack bioactive metabolites necessary for normal growth and survival. Inadequate bioactive metabolites and their precursors can lead to nutritional imbalances, physiological changes and diseases (Harrison 1990). Despite the commercial benefit of enhancing larval production by providing optimized maturation diets, insufficient research in this topic has been carried out. This article focuses on improving maturation of reproductive Argentine red shrimp broodstock through the use of bioactive substances like carotenes added to artificial diets to finally define its biological function as a dietary supplement.

Use of bioactive substances, such as nutritional additives, to improve the yields of cultured shrimp is a topic that has received increasing attention to define the biological function of carotenoids as a dietary supplement. Carotenes play an important role in organism health by acting as biological antioxidants, avoiding deterioration of cells and tissues. In crustaceans, they stimulate the immune system, serve as a source of vitamin A, increase resistance to diseases and enhance the rate of reproduction, weight gain and survival.

Penaeid shrimp cannot synthesize carotenes *de novo*, and consequently, these must be obtained through dietary sources. Variation in pigmentation from different sources can be attributed to the type, composition and concentration of the pigments contained in the diet, digestibility of the material and likely the presence of cofactors involved in absorption and deposition (Díaz *et al*. 2012).

Crustaceans have the ability to transform carotenoids into a limited range of closely related derivatives. Oxidative pathways suggested for metabolism of dietary carotenoids indicate that pure

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TABLE 1. INGREDIENT COMPOSITION OF REFERENCE DIET.

Ingredient	*g/100 g*
Fish meal	41.5
Squid meal	25.0
Clam meal	4.0
Soybean meal	4.5
Manioc starch	15.0
Fish oil	3.0
Fish soluble	3.0
Soy lecithin	1.0
Cholesterol	1.0
Vitamins	0.5
Na alginate	1.5
Proximate composition	*% dry matter*
Crude protein	54.5
Lipid	13.7
Ash	7.1
Moisture	7.0

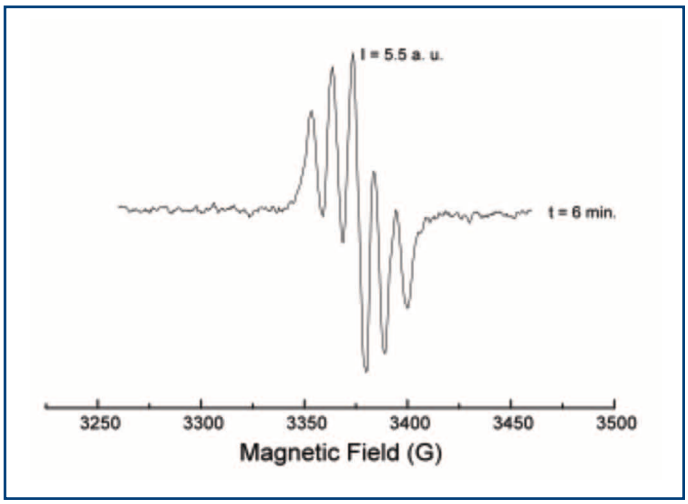
pigments, such as b-carotene, zeaxanthin and canthaxanthin, can be converted to astaxanthin (Tanaka *et al*. 1976). Astaxanthin attracted considerable interest because of its potent antioxidant activity and for its economic value as a pigment source in the aquaculture and food industries. Changes in the levels or activities of the main antioxidants have been proposed as biomarkers of health status in marine organisms.

Electron paramagnetic spin resonance (EPR) is a potent tool for determining the level of oxidative stress *in vitro* and *in vivo* (Díaz *et al*. 2004, Buico *et al*. 2008). The antioxidant activity of a molecule is measured by evaluating its ability to scavenge radicals. Because of their short lifetime, radicals cannot be detected directly by EPR in aqueous solution at room temperature, and they can only be measured using a spin-trapping agent. In this study, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was employed to measure antioxidant capacity.

Study Methods

Unilaterally-ablated immature females with an initial mean weight of 18.5 g were held in aerated circular tanks of 3 m diameter (33 g/L salinity, temperature 20-21°C, pH 7, 12:12 h photoperiod). Shrimp were maintained at 10/m^2. Two feeds with the same protein and lipid content were formulated and supplemented with the carotenoids astaxanthin and β-carotene, each at 300 mg/kg diet. A formulated diet without carotenoid supplementation served as control (Table 1).

After 60 days of experimentation, shrimp were dissected and ovaries, integument and midgut gland were extracted and lyophilized. Female specimens after one day (wild) and 7 days of acclimatization (initial) were also sampled as controls. Samples were homogenized under an argon atmosphere in darkness. Carotenoids were extracted and separated following the procedure of Schiedt *et al*. (1993). Non-polar carotenoids were extracted with



TOP AND BOTTOM, FIGURE 3. *UV-visible spectrophotometer (top) and EPR spectra of DPPH free radical (bottom).*

hexane and polar carotenoids with dimethyl sulfoxide/acetone. Absorption peaks of different carotenoids were identified by scanning the spectrum between 200 and 750 nm using an UV-visible spectrophotometer. Antioxidant activity of the midgut gland was investigated on the basis of the scavenging activity on the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH) free radical using spectroscopy of the EPR technique (Fig.3) (Díaz *et al*. 2004).

Broodstock Performance

Food is a critical factor to promote maturation in females of Argentine red shrimp under culture conditions, so a diversified diet covers most nutritional requirements. Mixed diets consisting of different natural fresh-frozen food and dried formulated feed are more efficient than a single food. This feeding regime has a synergetic effect on ablated specimens, improving female maturation in this species, with 50 percent success (Fig. 4; Díaz and Fenucci 2004).

Addition of carotene (as astaxanthin or β-carotene) increased

TABLE 2. Percentage of full mature females of Argentine red shrimp fed different diets.

Treatment	*% fully ripe females*
Control	75
Astaxanthin	100
β-carotene	100

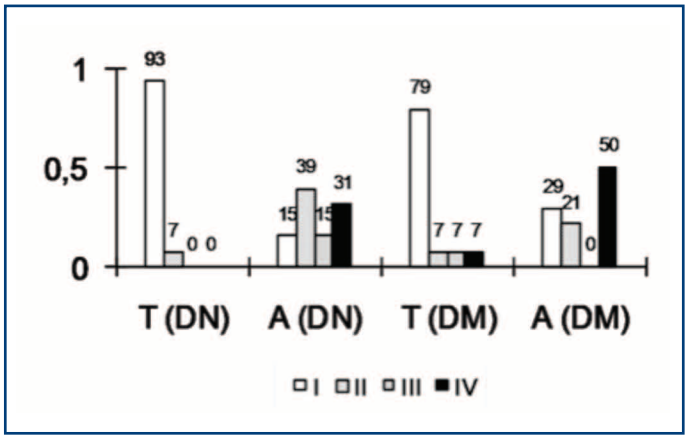


FIGURE 4. *Percentage of maturation stages of ablated (A) and non-ablated (T) females fed natural (DN) and mixed (DM) diets.*

the number of females that reached maturation stage IV compared to the control (Table 2). Ten females were stocked with males to obtain fertilization. The number of eggs produced by ablated females (163,000-368,000) corresponds to the fecundity of wild females (Macchi *et al*. 1992) and is similar to those obtained for other penaeid species. No correlation was found between the number of eggs spawned and the size of females (r=0.173). The hatching rate of fertilized eggs was between 72 and 99 percent.

Tissue Carotene Content

Availability of dietary carotene sources affects its content and concentration in crustaceans. In the tissues of mature wild females, a high carotene concentration was observed when compared with reared ones, probably because of the higher concentration and availability of carotenoids in the natural environment (Liñán-Cabello *et al*. 2003).

Tissue carotene analysis in Argentine red shrimp indicated that non polar carotenes (β-carotene) were more abundant than polar ones (free astaxanthin), both in integument and ovary. The smaller ratio of free astaxanthin to β-carotene can be related to the biotransformation (esterification) of free astaxanthin, becoming stored as lipid globules or forming complexes of carotenoproteins (Wade *et al*. 2005).

The concentration of total carotenes in the ovaries of Argentine red shrimp was greater than that of total carotenes found in integument, both in treated and wild animals (Table 3). Although the hypodermis and exoskeleton in crustaceans represent 60 to 90 percent of total pigments, significant quantities of pigments can be relocated during molting or maturation to

TABLE 3. Number of eggs and percentage of nauplii obtained from females of Argentine red shrimp.

Female weight (g)	*Number of eggs*	*Nauplii (%)*
28.2	270,000	84.6
25.6	347,775	99.3
29.0	368,750	80.0
28.4	163,000	80.8
32.2	268,500	93.1
22.5	239,000	75.0
29.6	228,800	39.3
35.0	350,000	72.0
30.9	260,000	83.0
29.9	195,000	78.0

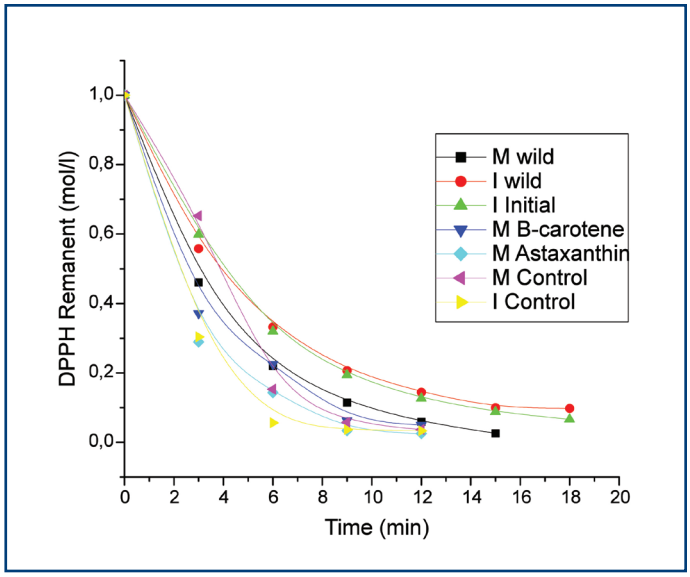


FIGURE 5. *Free radical scavenging activity of midgut gland tissues.*

other tissues such as haemolymph, midgut gland, gonads and eggs. For instance, during early maturation, free and esterified carotenes accumulate in the midgut gland but, during secondary vitellogenesis, they move from the midgut gland to the ovaries. These physiological processes generate highly reactive free radicals that are capable of destroying the integrity of cellular membranes, enzymes and nuclear DNA. Antioxidants, such as carotenoids, neutralize these highly reactive free radicals, protecting the structural and functional integrity of cells.

Antioxidant protective capacity in Argentine red shrimp, measured as the decay of the DPPH radical over time, was not significantly different among treatments (Table 4). Nevertheless, antioxidant protective capacity was observed in shrimp in all treatments (Fig. 5). The efficiency of carotenoids as antioxidants does not follow their capability as radical scavengers or the order of their oxidation potential (Han *et al*. 2006). The long-standing controversy of the function of carotenoids as antioxidants may be related to extrapolation of properties determined in homogeneous solutions to more complex biological systems, where other factors such as spatial organization and interaction among antioxidants becomes important.

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TABLE 4. Concentration of carotenoids non polar and polar in integument and ovaries (µG/G tissue) (mean ± SD, n=3).

		Non-polar		*Polar*	
		Integument	*Ovary*	*Integument*	*Ovary*
Wild	I	1.63±0.38b	2.42±0.20c	0.68±0.08a	ND
	M	0.80±0.22a	5.93±0.66d	0.59±0.04a	5.37±0.78b
Initial	I	1.68±0.62b	3.88±0.17e	1.05±0.09a	0.90±0.23c
Control	I	2.03±0.26c	2.44±0.00e	1.59±0.09a	ND
	M	1.93±0.39c	5.32±0.52d	1.11±0.30a	2.12±0.36d
Astaxanthin	M	2.50±0.55c	3.19±0.55e	0.80±1.12a	2.03±0.50d
β-carotene	M	2.04±0.20c	5.35±1.30d	0.46±0.06a	3.17±0.50d

Means in a row with different superscripts differ significantly (p<0.05).ND: undetected; M: mature; I: immature.

Implications

Because the life cycle of Argentine red shrimp occurs entirely in the marine environment, the capture of large numbers of postlarvae in coastal areas for grow-out is not feasible. For this reason, it is important to know details of maturation, mating and especially the nutritional requirements of this species. Our research group has successfully completed maturation, mating in captivity, mass larval culture until PL20 and grow-out in ponds from juvenile to commercial size (20-27 g) in 140 days. More research is needed to obtain juveniles of 1 g in one month or less.

Notes

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