Disposition of Carotenoids in the Blue Goose Barnacle *Lepas fascicularis*[1]

An earlier note[2] included a discussion of the oceanic habitat and occasional springtime washing to shore of the blue, pelagic goose barnacle *Lepas fascicularis* by steady on-shore winds. It reported also the results of a preliminary analysis (based on a single available specimen) which showed astaxanthin to be the preponderant carotenoid present, accompanied by secondary amounts of neutral carotenoids exhibiting spectral properties characteristic of β-carotene, but doubtless inclusive of xanthophyllic derivatives thereof as well.

In that earlier report, it was anticipated that a future spring season should provide an opportunity to complete a better analysis with the anticipated availability of more of the fresh specimens. Fortunately, such an opportunity returned early in May of 1967, when countless thousands of the blue siphonophore *Velella lata* were borne shoreward and lay in windrows on the beach of the Scripps Institution, as well as to the north and south of this locality. The *Velella* were accompanied by limited numbers of *Lepas fascicularis* and many more *L. pectinata pacifica*, both of which were wind-rafted to shore either free-floating (in the instance of the former) or both species attached to *Velella* floats, floating feathers, sticks of wood or fragments of tar.

Lepas fascicularis exhibits various shades of blue, notably in the cirri, carapace and the ripe ovary within the peduncle, which appears dark blue at sexual maturity.

Thirty-one fresh specimens, chilled to achieve maximal relaxation for dissection, varied in carapace length from 7 (in a few instances) through about 14 (in most) to 27 mm. The spongy, partially gas-laden, sand-covered floats, which were pale tan in color, were dissected away and discarded. Of the inner tissues the thoracic wall was pigment-free, the spermaries brown-walled, and the alimentary tract dark brown, full of degraded food materials, but with considerable blue pigment within associated tissues. The cirri, trophi, carapace and ripe ova carried most of the blue pigment, which rapidly turned to pink or orange on exposure to ethanol, dissolving therein to give yellow or orange extracts.

Whole gut tissues were separated from the rest of the somatic tissues, blotted on paper, weighed wet (5.79 g), placed in ethanol under nitrogen, stored in a refrigerator and agitated occasionally to facilitate complete extraction of the carotenoids. The same procedure was followed with the residual somatic tissues (some including immature eggs), which aggregated a total wet weight of 10.97 g. Six of the animals showed deep blue pigmentation of the ovaries as viewed through the wall of the peduncle. From these animals an aggregate of 0.53 g (wet weight) of eggs was recovered through the cut distal end of the stalk. A small sample of eggs was completely shattered, on centrifugation in sea water, yielding minute spherules of blue chromoprotein in the bottom of the tube and colorless oil droplets of larger sizes near the surface of the water. The intact eggs were deep blue prolate spheroids (108×156 μ) without microscopically visible yolk bodies. They were extracted from all carotenoid material with cold ethanol.

Reagents, spectrophotometric and thin-layer chromatographic equipment used in this work have been described and illustrated previously[3]. Ethanolic extracts of carotenoids were filtered free of tissue residues by drawing through celite. All carotenoid material was transferred to hexane, wherein readings were recorded with a Bausch & Lomb Spectronic 505 recording spectrophotometer.

For thin-layer chromatography we employed Silica Gel (E. Merck, Darmstadt, Germany; distributed by Brinkman Instruments Inc., N.Y.), and 20–30% acetone in hexane as eluant. Carotene was distinguished from epiphasic xanthophyll esters by its persistent epiphasic behavior after treatment with alcoholic NaOH and reapplication of the partition test in hexane versus 95% methanol. Astaxanthin was characterized by its single rounded absorption maximum at 470 nm in hexane, its strongly hypophasic behavior in the partition test, and its ready convertibility into astacene as a soap thereof on warming in air in the presence of mild alkali. A summary of the analytical findings follows (Tables I, II and II).

There were traces of yellow pigment between fractions 2 and 3, and between fractions 3 and 4 (Table II); also a trace of green material (probably from chlorophyll degradation) between the third and fourth zone. The relatively high proportions of algal carotenoids, e.g. β-carotene, free and esterified zeaxanthin, some other free, unidentified neutral xanthophylls, and notably peridinin (11.70%) with traces of green pigment, offer inferential evidence for the representation of algae and algal detritus in the barnacle's natural diet.

Table I. Eggs. Concentration of total carotenoids (in astaxanthin units): 5.1 mg/100 g wet weight

Fraction No. (color)	λ_{max} (nm)	Compound (partition)	% of total
1 (yellow)	like β-carotene	Zeaxanthin epiphasic esters (100/0)	2.05
2 (pink)	470	Astaxanthin ambiphasic esters (28/72)	5.15
3 (pink)	470	Astaxanthin	70.50
4 (pink)	470	Astaxanthin	22.10

Table II. Whole gut. Concentration of total carotenoids (in astaxanthin units): 2 mg/100 g wet weight

Fraction No. (color)	λ_{max} (nm)	Compound (partition)	% of total
1 (yellow)	like β-carotene	β-carotene (100/0)	6.47
2 (yellow)	like β-carotene	Zeaxanthin esters (100/0)	1.95
3 (yellow)	like β-carotene	Zeaxanthin esters (70/30)	2.32
4 (pink)	469	Astaxanthin (14/86)	8.62
5 (pink)	470	Astaxanthin (11/89)	47.30
6 (yellow)	~472; 453; 417	Axanthophyll (9/91)	14.14
7 (yellow)	~474; 446; 428	Axanthophyll (8/92)	7.37
8 (pink)	483; 454; 430 (468 in methanol)	Peridinin (~0/100)	11.70

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Lepas fascicularis is typical of some pelagic crustaceans in the predacious equipment of its thoracic appendages, specialized for capturing and tearing or crushing small organisms; finely particulate detrital matter is also derived from its environment[4]. Examination of its gut contents characteristically revealed many remains of copepods, accompanied by much yellow-brown material of a finely microscopic or colloidal nature, while more of the latter material seemed to be stored in the dark brown digestive diverticula. The carotenoid analysis of the whole gut, including food materials, revealed minor proportions of β-carotene (ca. 6.5%) and zeaxanthin (ca. 4.3%), but fully $^1/_3$ of the total carotenoids as other free, neutral xanthophylls, including the typical dinoflagellate compound, peridinin (11.7%), while the remainder, approximately 56%, was astaxanthin.

It may hardly be doubted that *Lepas* resembles many other Crustacea in the ability to oxidize plant carotenoids to astaxanthin, subsequently storing it free, or esterified, or conjugated as chromoproteins. Still, it may also derive some astaxanthin as such from consumption of copepods.

The eggs were richest in carotenoids, i.e. 5 mg, as compared with 2 mg in the gut and 1 mg/100 g in residual somatic tissues and carapace. The proportion of astaxanthin also was highest in eggs, comprising therein 97.75% versus 82.2% in residual soma, and but 56% in gut and contents.

It would appear that virtually all of the astaxanthin in the ova was conjugated as blue chromoprotein, since the centrifugation experiment demonstrated the separability of blue spheroid micelles of relatively high density from colorless, supernatant oil droplets. There was no carotene in the ova, and but 2% of the total carotenoid therein was esterified zeaxanthin. The separated oil droplets appeared to be colorless, both when seen as aggregates in the centrifuge tube and when viewed through the microscope, hence hardly could have carried any of the astaxanthin in dissolved condition.

It is suggested that the storage of astaxanthin-protein complexes in the eggs, which contain no organized yolk mass, may serve certain alternative but not necessarily mutually exclusive functions, e.g. the carotenoid may, in some way, serve to stabilize the protein against thermally or photically induced modification in warmer seas, where the animals usually live (cf. the astaxanthin chromoprotein, ovorubin, in the eggs of the gastropod *Pomacea canaliculata*, which appears to serve such a role when the eggs are deposited out of water, exposed to the direct rays of the sun[5]). Or the arrangement may serve to 'pack in' relatively large amounts of the astaxanthin, in a finely dispersed condition, rather than in oil droplets, thus rendering both the carotenoid and the protein moiety available for some role to be satisfied in growth or developmental processes.

HERRING[6] has suggested the possible vulnerability of either unconjugated moiety of a carotenoid-protein complex to high light-intensities or high temperatures, and the release of egg pigments for metabolic use during development. His alternative proposal, relating to the implementation of some degree of chromatic concealment by a blue chromoprotein in blue oceanic waters, would appear to find less support in the instance at hand. For the ripe eggs of *L. fascicularis*, showing their distinct blue color through the wall of the peduncle, are not released but, on internal fertilization, gradually lose their blue pigmentation, leaving the peduncle pale as they develop and hatch as nauplii. Barnacle larvae are colorless, save for their pigmented eyes, and are carried within the mantle cavity until released as free-swimming food-consumers.

No tests were applied for the presence of preformed vitamin A, but no precursor to the vitamin, such as one of the carotenes or cryptoxanthin, was detected in the eggs. It is conceivable that the rich stores of astaxanthin may serve, in part at least, for the elaboration of the animal's photoreceptive equipment, as it seems to do in locusts[7], marine microcrustaceans[8–11], sea-stars[12] and other invertebrates[13]. It may also serve other metabolic roles, such as electron transport, enzymic activity, development[14], and other functions fulfilled by A vitamins in higher animals.

Zusammenfassung. Die blauen ozeanischen Cirripedien, *Lepas fascicularis*, speichern Karotinoide in Eiern, Eingeweiden und den meisten übrigen Geweben in den folgenden ungefähren Konzentrationen: 5:2:1 mg/100 g. In jeder Art von Gewebe ist Astaxanthin das Hauptkarotinoid, welches meist in Form des blauen Chromproteins vorliegt. Kleinere Mengen von Karotin und etwas mehr Xanthophylle kommen in den Eingeweiden, nicht aber in den reifen Eiern vor.

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Table III. Blue somatic tissues, plus ripening eggs. Total carotenoids (astaxanthin units): ca. 1.06 mg/100 g wet weight

Fraction No. (color)	λ_{max} (nm)	Compound (partition)	% of total
1 (yellow)	~473; 448	β-carotene (100/0)	4.23
2 (yellow)	474; 449; ~425	Zeaxanthin esters (100/0)	1.64
3 (yellow)	475; 450; 425	Zeaxanthin esters (ambiphasic)	2.83
4 (orange-pink)	470	Astaxanthin (13/87)	47.70
5 (orange-pink)	470	Astaxanthin (9/91)	34.50
6 (yellow)	~474; 452	Contaminated xanthophyll (hypophasic)	4.45
7 (yellow)	474; 447	Zeaxanthin (hypophasic)	4.34

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