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Carotenoids in the gonad and gut of the edible sea urchin *Psammechinus miliaris*

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ABSTRACT

The carotenoid content and composition of the gonad and gut of the edible sea urchin *Psammechinus miliaris* were determined. The dominant carotenoid of the gut wall was fucoxanthinol, indicating the degradation of the main carotenoid found in the Phaeophycean algae. Also present in the gut wall was the carotenoid echinenone, a pigment absent from the diet of wild urchins. Its presence in the gut wall indicates that this is a site for metabolism of dietary, algal, β-carotene. Fucoxanthinol and related products are not found in the gonad and instead this organ was found to selectively accumulate echinenone and β-carotene together with a small number of related compounds. Both echinenone and β-carotene were found in two geometric forms, namely all-*trans* and 9′-*cis* or 9-*cis* respectively. Echinenone is predominantly deposited in the gonad as the 9′-*cis* form, whilst β-carotene is mainly found in the same form as that seen in the algae that, in part, make up its diet in the wild, i.e., all-*trans*. This indicates that isomerisation of dietary β-carotene occurs as part of the process of accumulating echinenone in the gonad. The colour and overall appearance of the gonads of this wild and food limited population were generally poor. No correlation between CIE *L*a*b* chromameter readings or a visual assessment of gonad colour with carotenoid content as determined by HPLC was observed.

1. Introduction

Psammechinus miliaris is a regular echinoid, found all around the British Isles but locally abundant in sheltered sea lochs on the west coast of Scotland (Kelly et al., 2007). It has an annual reproductive cycle, the gonad index (G.I.) peaking in the June and July coinciding with the onset of the spawning period (Kelly, 2000). In natural populations, if food is limited, the gonad regresses after the spawning period and typically remains of low biomass until the following spring (Kelly, 2000; Hughes et al., 2006).

Many species of sea urchins are valued for their edible roe (the term used to describe the gonads of both sexes), however many populations are now over fished (Andrew et al., 2002) and culture methods are being developed as a consequence. Although smaller in test diameter than *Paracentrotus lividus*, commonly sold for human consumption in Europe, *P. miliaris* has a pleasantly flavoured roe which increases in biomass rapidly when the urchins are provided with ample nutrition (Cook et al., 1998). *P. miliaris* has been shown to be robust in culture (Kelly, 2002), can be routinely raised from the larval phase in the hatchery (Kelly et al., 2000) and has been shown to thrive when maintained in polyculture with Atlantic salmon (Kelly et al., 1998). As such it has potential as a candidate aquaculture species although formulated diets that enhance both gonad biomass and colour are still required, as a bright orange or yellow gonad colour is critical for obtaining best prices.

Gonad colouration in sea urchins is a result of the deposition of carotenoids obtained from their diet. Both direct deposition of these carotenoids (without modification) and deposition of carotenoid metabolites has been observed (Tsushima and Matsuno, 1990; Symonds et al., 2007). The dominant carotenoids in the gonads of sea urchins are typically echinenone (both α- and β-forms: β,ε-caroten-4-one and β,β-caroten-4-one, respectively and β-carotene (β,β-carotene); Fig. 1) and β-carotene. The major carotenoid from the fronds of brown algae, namely fucoxanthin (5,6-epoxy-3′-ethanoyloxy-3,5′-dihydroxy-6′,7′-didehydro-5,6,7,8,5′,6′-hexahydro-β,β-caroten-8-one) often with a metabolite, fucoxanthinol (5,6-epoxy-3,3′,5′-trihydroxy-6′,7′-didehydro-5,6,7,8,5′,6′-hexahydro-β,β-caroten-8-one; Fig. 1), has often been reported in abundance in whole soft tissue sea urchin extracts. In a recent study (Symonds et al., 2007) the carotenoid pigments of *P. lividus*, sympatric with *P. miliaris* over part of its range, were characterised. The major carotenoids detected in the gut wall were breakdown products of fucoxanthin, namely fucoxanthinol and amarouciaxanthin A (3,5,6′-trihydroxy-6,7-didehydro-5,6,7′,8′-tetrahydro-β,ε-carotene-3′,8′-dione). Lower levels of other dietary carotenoids, lutein (β,ε-carotene-3,3′-diol) and β-carotene, and non-dietary carotenoids, isozeaxanthin (β,β-carotene-4,4′-diol) and echinenone (approximately 20% total carotenoid), were also detected in the gut wall. The presence of echinenone in the gut wall demonstrated that in this species this organ acts as a major site of carotenoid metabolism. Echinenone was the dominant carotenoid in the gonads, accounting for approximately 50-60% of the total carotenoids. Both all-*trans* and 9′-*cis*

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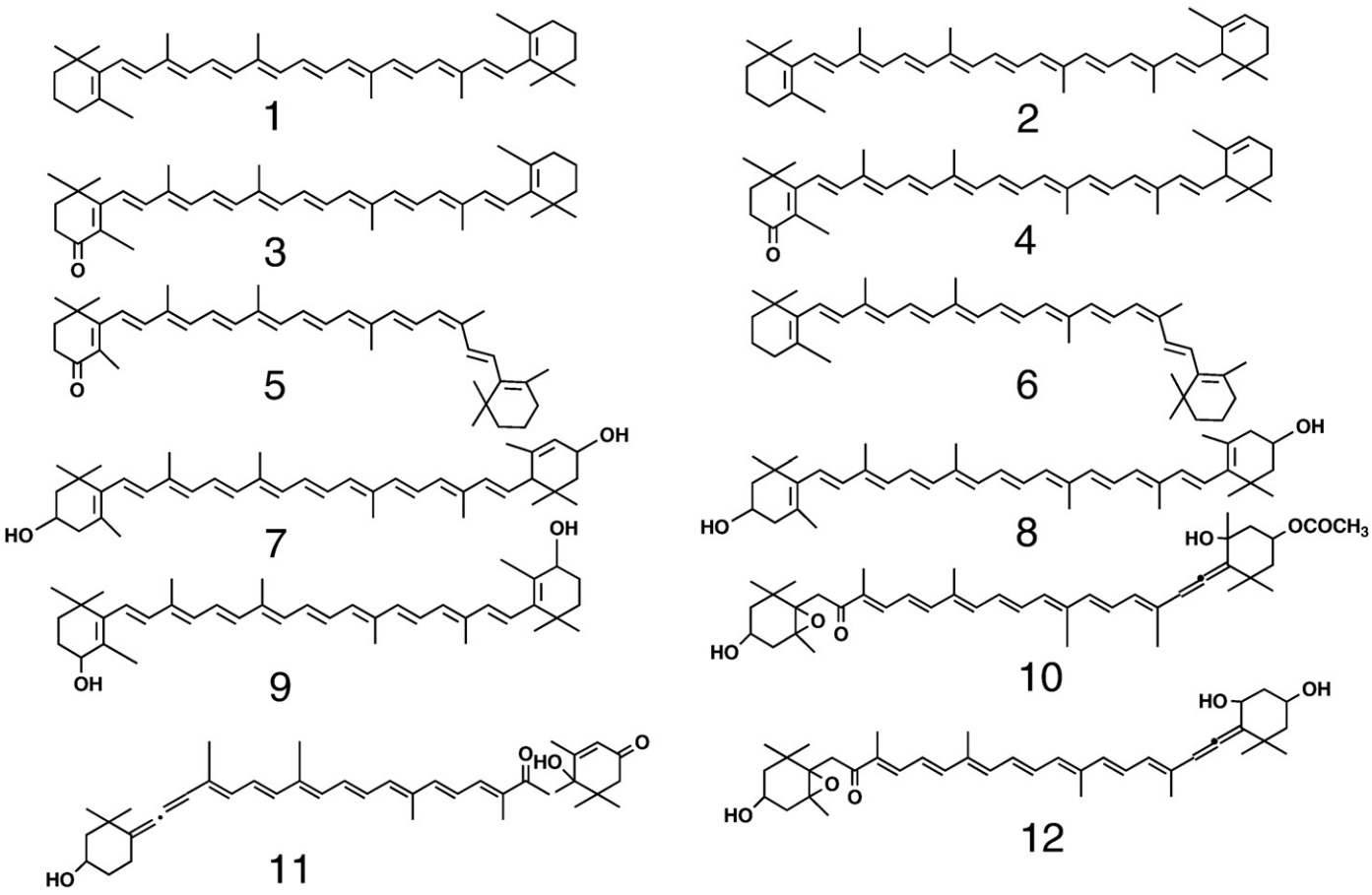

Fig. 1. Structures of carotenoids. 1. all-*trans* β-carotene, 2. all-*trans* α-carotene, 3. all-*trans* echinenone, 4. all-*trans* α-echinenone, 5. 9′-*cis* echinenone, 6. 9-*cis* β-carotene, 7. lutein, 8. zeaxanthin, 9. isozeaxanthin, 10. fucoxanthin, 11. amarouciaxanthin A, 12. fucoxanthinol.

forms of echinenone were detected in both the gut wall and in the gonad, with levels of the 9′-*cis* form typically 10-fold greater than the all-*trans* form in the gonad. These authors concluded that, for the wild population studied, visual assessment of gonad colour was not a good proxy for the levels of total carotenoid or echinenone in the gonad and that other unidentified factors were also playing a role in the visual assessment or appraisal of gonad colouration. Conversely, however, in a study of *P. lividus* fed experimental diets Shpigel et al. (2006) found dietary β-carotene, gut β-carotene and gonad echinenone levels had a positive correlation with acceptable (marketable) gonad colour. It should be noted that although the urchins in both studies had a similar G.I. (8.0–11.5%), the urchins in the study of Symonds et al. (2007) were approximately 32 g greater in total wet weight.

The objective of the present study was to detail the carotenoid profiles of the gonad and gut of two inter-tidal *P. miliaris* populations from a Scottish sea loch. Urchins from these locations had been intensively studied previously to determine the periodicity of the reproductive cycle (Kelly, 2000) and to determine the links between seasonal changes in gonad fatty acid biochemistry and diet (Hughes et al., 2005, 2006). The aim was to gain a better understanding of how carotenoids may influence gonad colour, and estimate temporal variation in carotenoid content and any changes that may be linked to reproductive state or gender.

2. Methods

2.1. Sample collection

Approximately ten *P. miliaris* were collected quarterly from each of two intertidal sites, 50 m apart, at Rubha Garbh, Loch Creran, west coast of Scotland from January to September 2006 (n=73 in total).

Table 1
Gonadal index (G.I.), alimentary index (A.I.), quantitative colour measurements (CIE L^*a^*b), colour market acceptability (EAU) and number (n) of wild *P. miliaris* samples collected from January–September 2006, male and female samples combined

Sample month	n	G.I. (%)	A.I. (%)	CIE L^*	CIE a^*	CIE b	E %	A %	U %
January	20	♂: 0.65±0.09	1.56	20.87	3.64	8.43	0	0	100
		♀: 1.02±0.29	±0.17	±0.98	±0.44	±1.18			
April	18	♂: 1.11±0.29	2.21	24.66	2.87	11.02	11	0	89
		♀: 2.68±0.49 s	±0.32 s	±1.63	±0.65	±2.17			
July	20	♂: 3.94±0.68 s	1.73	26.73	4.01	10.72	5	5	90
		♀: 3.36±0.26	±0.13	±26.73	±0.43	±1.11			
September	15	♂: 1.13±0.23	1.73	25.30	3.68	6.84	0	0	100
		♀: 1.38±0.13	±0.25	±0.87	±0.48	±1.17			

s = Significant differences.