Carotenoids in Wild and Cultured, Red Sea Bream, Pagrus major TEMMINCK & SCHLEGEL and Prawn, Penaeus japonicus BATE.

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Carotenoids in Wild and Cultured, Red Sea Bream, *Pagrus major* Temminck & Schlegel and Prawn, *Penaeus japonicus* Bate.

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Abstract

In order to improve red color tone of cultured aquatic animals, the carotenoids in wild and cultured red sea bream, *Pagrus major* Temminck & Schlegel and prawn, *Penaeus japonicus* Bate were compared. The main carotenoid in both red sea bream and prawn was astaxanthin and its amounts were conspicuously different between wild and cultured animals.

In order to improve the red color of red sea bream, a feeding experiment of spheroidenone producing bacteria and sea mussel was performed. The color of red sea bream was not improved by feeding spheroidenone, but red sea bream fed on sea mussel demonstrated fairly improved coloration and the carotenoids in sea mussel were deposited in the integuments of red sea bream.

The constituents of the carotenoids in marine red fish such as sea bream were reported, and the main carotenoid of these fish is astaxanthin[1]. The feeding experiment of β-carotene, canthaxanthin and zeaxanthin shows that the fish could not convert these carotenoids to astaxanthin. In other words, sea bream cannot oxidize the 3, 3′ and 4, 4′ positions of the β-ionone rings[1].

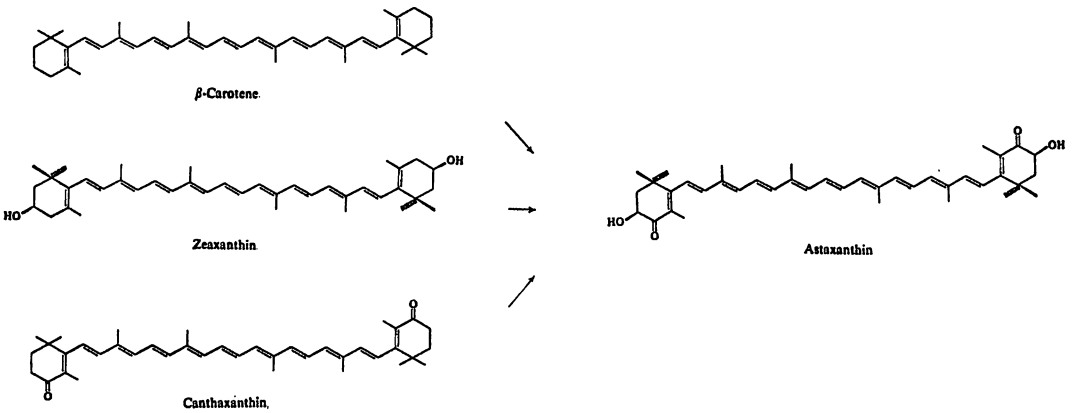

Fig. 1 Precursor of astaxanthin in tigar prawn, *Penaeu japonicus* Bate.

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The relative abundances of the carotenoids obtained a number of species of crustacea were reported by the authors[2], astaxanthin was by far the most prominent carotenoid and the most important pigment like the case of sea bream. It was clarified, however that prawn could convert β-carotene, canthaxanthin and zeaxanthin to astaxanthin[3]. (Fig. 1). In other words, prawn can oxidize the 3, 3′ and 4, 4′ positions of the β-ionone rings.

In this experiment, the contents of wild prawn and red sea bream were compared with those of faded, cultured sea bream and prawn. Spheroidenone producing bacteria and sea mussel were fed to sea bream in order to improve their color.

Materials and Methods

1. Comparison of carotenoids in wild and cultured, prawn and red sea bream:

Wild prawn, *Penaeus japonicus* BATE were purchased at a local fish market and cultured prawn were obtained from the Mitsui Norin prawn farm. Wild red sea bream, *Pagrus major* TEMMINCK & SCHLEGEL were purchased at a local fish market and culturd sea bream were obtained from the Nagashima Marine Laboratory Fisheries Science of our Faculty.

In this experiment, the integuments of red sea bream were extracted with acetone in a Waring blender until no further pigments were obtained. The acetone solutions were combined, transferred to petroleum ether by addition of water and washed repeatedly with water to remove traces of acetone. The

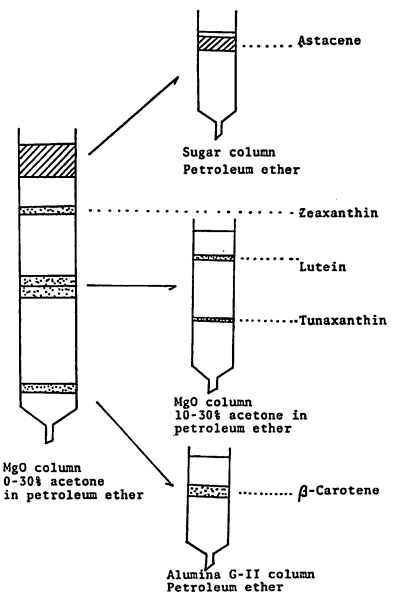

Fig. 2 Separation of carotenoids in red sea bream.

petroleum ether solution of the pigments was dried over anhydrous sulphate and concentrated under vacuum. Thus obtained crude carotenoids were saponified by dissolving it in 20 cc of absolute ethyl alcohol, adding 10 cc of 60 per cent aqueous potassium hydroxide (w/v) and leaving it overnight under nitrogen at room temperature. The saponified pigments were transferred to petroleum ether with water, dried with anhydrous sodium sulphate and chromatographed.

The carotenoids in red sea bream were initially separated on a column of magnesium oxide as shown in Fig. 2. The carotenoid pigments of each band were purified on magnesium oxide, alumina and sugar columns. The carotenoids were characterized by their behaviors on the columns, their absorption spectra and co-chromatography with authentic samples. The quantitative determination was carried out mathematically by extinction value at the maximum absorption.

The whole body of prawn were extracted with acetone. The separation procedure of the carotenoids in prawn is shown in Fig. 3. Identification and quatitative determination of the carotenoids in prawn are similar to those in red sea bream.

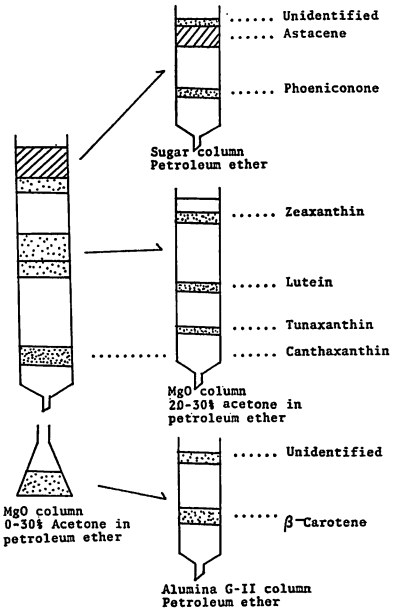

Fig. 3 Separation of carotenoids in tigar prawn.

2. Feeding experiment of spheroidenone producing bacteria to red sea bream:

Alive and healthy red sea bream were obtained from the Nagashima Marine Laboratory for Fisheries Science of our Faculty. Two aquaria were prepared. Fifteen red sea bream (about 50 g) each were placed in each tank and acclima-

ted for two weeks to the artificial diet[4]. The fish in the two tanks were fed every day for 32 days as follows: Tank 1-artificial diet (control); Tank 2-control diet + 20% spheroidenone producing bacteria. The constituents of the carotenoids in the spheroidenone producing bacteria is shown in Table 1.

Table 1. Relative abundance of the carotenoids in spheroidenone producing bacteria.

Carotenoids	Relative abundance (%)
Spheroidenone	96.5
Spheroidene	1.4
Unknown	2.1

3. Feeding experiment of sea mussel to red sea bream:

The constituents of the carotenoids in sea mussel is shown in Table 2. Sea bream were separated into two groups. Thirty each of one group was fed with artificial diet and thirty each of the other group was fed with artificial diet + 50% sea mussel every day for 32 days. The separation and identification of the carotenoids in this test fish were carried out as the same way already stated.

Table 2. Relative abundance of the carotenoids in *Mytilus edulis*.

Carotenoids	Relative abundance (%)
β-Carotene	10.1
Lutein	5.2
Zeaxanthin	10.9
Isomytiloxanthin	34.7
Mytiloxanthin	26.6
P-451	5.0
Unknown	5.0

Results and Discussion

1. Comparison of the carotenoids in wild and cultured, prawn and red sea bream:

The contents of the carotenoids in wild and cultured prawn are shown in Table 3. As shown in Table 3, total carotenoids in wild prawn are quadruple as much as those in cultured ones and the amounts of astaxanthin in wild prawn are three times as much as those of cultured ones. From the external observation, wild prawn have reddish brown, beautiful stripes, but cultured ones

Table 3. Amounts and relative abundances of carotenoids in wild and cultured prawn.

Carotenoids	Wild μg/g	%	Cultured μg/g	%
Total carotenoid	81.93		21.62	
Astaxanthin	60.30	73.6	19.63	90.8
Canthaxanthin	10.08	12.3	0.56	2.6
Phoenicoxanthin	7.29	8.9	—	
Zeaxanthin	0.90	1.1	0.35	1.6
Tunaxanthin	0.82	1.0	—	
Lutein	0.49	0.6	0.26	1.2
β-carotene	0.25	0.3	0.24	1.1
Other carotenoids	1.80	2.2	0.58	2.7

Ten prawns (25-35 g) were used for each analysis.

assume rather blue color. The major cause for this difference of color tones comes from the contents of astaxanthin. As already stated, β-carotene, echinenone, canthaxanthin and zeaxanthin can be metabolized to astaxanthin. Therefore, those carotenoids are now being used as the sources of astaxanthin in order to improve their color at some prawn farms.

The contents of carotenoids and their constituents in wild and cultured red sea bream are shown in Table 4. As shown in Table 4, the contents of total carotenoids, especially the contents of astaxanthin in wild red sea bream are overwhelmingly much more than those in cultured ones. From the external observation, wild red sea bream appear bright red, but cultured ones appear grey black. It is assumed that this grey black color is attributable to melanophore. In this experiment, the existence of astaxanthin in cultured red sea bream was confirmed, but sometimes it is difficult to find it. As already stated, sea bream cannot convert β-carotene, canthaxanthin and zeaxanthin to astaxanthin, but they can deposit them in their integuments without modification. Therefore, in order to improve their color, if sea bream are to be cultured in

Table 4. Amounts and relative abundances of carotenoids in wild and cultured, red sea bream.

Carotenoids	Wild μg/g	%	Cultured μg/g	%
Total carotenoid	3.34		0.73	
Astaxanthin	2.32	69.5	0.07	9.6
Tunaxanthin	0.64	19.2	0.61	83.6
Lutein	0.19	5.7	0.02	2.8
Zeaxanthin	0.11	3.3	—	
β-carotene	0.02	0.6	—	
Other carotenoids	0.06	1.8	0.03	4.1

Ten fish (40-60 g) were used for each analysis.

an aquaculture program, astaxanthin or similar carotenoids must be included in the diet.

2. Feeding experiment of spheroidenone producing bacteria:

Table 5 lists the results of feeding red sea bream on an artificial diet supplemented with spheroidenone producing bacteria. As shown in Table 5, spheroidenone was not found in the integuments of the test red sea bream and the amounts of astaxanthin in the integuments were decreased. From external observation, there was no difference between the control group and the test one as far as color concerns.

Table 5. Amounts of the carotenoids in red sea bream fream fed on the diet containing spheroidenone producing bacteria.

Pigments \ Diet	Control diet	Diet containing 20 % spheroidenone producing bacteria
Astaxanthin found (μg/g body wt.)	0.074	0.044
Lutein found (μg/g body wt.)	0.053	0.044
Tunaxanthin found (μg/g body wt.)	0.357	0.530

3. Feeding experiment of sea mussel:

Table 6 records the results of feeding red sea bream on an artificial diet supplemented with sea mussel. This results show that mytiloxanthin and p-451 were found in the integuments of the test red sea bream. From external observation, the test group assumes bright orange-red color.

These results and those of previous studies show that sea bream cannot convert any carotenoid in diet to astaxanthin, but they can transfer those carotenoids whose end group is β-ionone ring, can be deposited in their integuments without modification.

Table 6. Amounts of the carotenoids in red sea bream fed on Mussel.

Pigments \ Diet	Control diet	Mussel
Astaxanthin found (μg/g body wt.)	0.038	0.050
Lutein found (μg/g body wt.)	—	0.032
Tunaxanthin found (μg/g body wt.)	0.086	0.034
Mytiloxanthin found (μg/g body wt.)	—	0.181
P-451 (μg/g body wt.)	—	0.179
Unknown (μg/g body wt.)	—	0.124

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Referances

1) Y. TANAKA, T. KATAYAMA, K. L. SIMPSON and C. O. CHICHESTER: The biosynthesis of astaxanthin-XX, The carotenoids in marine red fish and the metabolism of the carotenoids in red sea bream, *Chrysophrys major* TEMMINCK and SCHLEGEL ., *Bull. Jap. Soc. Sci. Fish.*, **42**, 1177-1182, 1976.

2) Y. TANAKA, H. MATSUGCHI, T. KATAYAMA, K. L. SIMPSON and C. O. CHICHESTER: The biosynthesis of astaxanthin-XVI, The carotenoids in Crustacea., *Comp. Biochem. Physiol.*, **42 B**, 263-264, 1972.

3) Y. TANAKA, H. MATSUGUCHI, T. KATAYAMA, K. L. SIMPSON andC. O. CHICHESTER: The biosynthesis-XVIII, The metabolism of the carotenoids in the prawn, *Penaeus japonicus* BATE, *Bull. Jap. Soc. Sci. Fish.*, **42**, 197-202, 1976.

4) T. KATAYAMA, T. KAMATA, M. SHIMAYA, O. DESHIMARU and C. O. CHICHESTER: The biosynthesis of astaxanthin-VIII, The conversion of labelled β-carotene-15, 15-$^3H_2$ into astaxanthin in prawn, *Penaeus japonicus* BATE., *Bull. Jap. Soc. Sci. Fish.*, **38**: 1171-1175, 1972.