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**Deterioration of Chum Salmon *Oncorhynchus keta* Muscle  
during Spawning Migration**

**XIV. Carotenoids in the serum lipoproteins of chum  
salmon associated with migration**

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**Abstract**

Carotenoids-carrying lipoproteins were studied using the sera of upstream migrating and spent chum salmon. Density gradient ultracentrifugal analysis on the serum confirmed that the carotenoids were associated with lipoproteins. There were two types of lipoproteins; high density lipoprotein (HDL,  $d=1.16$  g/ml) and very high density lipoprotein (VHDL,  $d=1.22$  g/ml) fractions, with orange coloration in the upstream migrating female serum, while only a colored HDL ( $d=1.16$  g/ml) fraction was obtained from the others. The HDL fraction from the spent male was yellowish, although that from the others was orange. The orange coloration from HDL and VHDL fractions was derived from astaxanthin. Three carotenoids other than astaxanthin were clearly detected from the HDL fraction of spent male. The water soluble yellowish pigments, possibly bilirubin, were present in the spent male HDL fraction. These results suggested that carotenoids-carrying lipoprotein from the HDL fraction could transport bilirubin as well as carotenoids other than astaxanthin.

**Introduction**

Pacific salmon, *Oncorhynchus* spp., are typical anadromous fish that grow in the ocean and breed in fresh water. This biological feature governs the pattern of changes in their chemical composition.<sup>1)</sup> For example, after migrating chum salmon enter a river, the muscle discolors and the integument becomes dark yellow or red. These changes in muscle or integument color may be closely associated with the physiological state of the fish which is probably controlled by sex steroids.<sup>2-7)</sup>

Kitahara<sup>8)</sup> has reported that carotenoids, mainly astaxanthin, in chum salmon muscle are transported into the integument and gonads *via* the blood serum. Little has been reported concerning carotenoids-carrying lipoproteins in the serum of salmon, although several studies on the distribution and characterization of serum lipoproteins in salmon have been made.<sup>9-12)</sup> Nakamura *et al.*<sup>13,14)</sup> have recently found that astaxanthin in the serum of the upstream migrating chum salmon is exclusively transported by high density lipoprotein. In previous papers,<sup>15-19)</sup> it was demonstrated that various serum lipoproteins such as high density lipoprotein and very high density lipoproteins including vitellogenin transport muscle astaxanthin into the integument and ovaries during spawning migration, and further, a scheme has been proposed regarding carotenoids metabolism in chum salmon.

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During the studies on nuptial coloration of chum salmon it was noticed that carotenoids-carrying lipoproteins had the ability to transport not only astaxanthin but also other carotenoids.<sup>15,17)</sup> The present paper describes the serum lipoproteins with carotenoids other than astaxanthin.

### Materials and Methods

#### Materials

Specimens of chum salmon, *Oncorhynchus keta*, in two physiological states were used as the materials (Table 1). Blood was collected from the caudal vasculature of live salmon at each migration stage, and left at room temperature for several hours. The clotted blood was centrifuged to obtain the serum. Sera thus obtained were stored at  $-20^{\circ}\text{C}$  until use.

#### Preparation of lipoproteins

The serum was analyzed by density gradients.<sup>20)</sup> An equal amount of 0.75% NaCl was gently layered over 4.75 ml serum containing 1.9 g of KBr, and centrifuged at  $218,000 \times g$  at  $10^{\circ}\text{C}$  for 5 hr. At the end of a run, the tubes were removed from the rotor and seven fractions were collected from each tube by pipette.

#### Analysis of fractions

The levels of proteins and carotenoids in each fraction were measured. Protein was measured by the biuret method,<sup>21)</sup> using bovine serum albumin as the standard. Carotenoids extraction was done with ethanol-diethyl ether. The carotenoids level was calculated, assuming the  $E_{cm}^{\%}$  value in ethanol at 480 nm to be 2,200. Absorption spectra were measured with a Hitachi 556 double wavelength spectrophotometer. The refractive index of each fraction was measured with an Abbe refractometer (Atago Co., Tokyo) and the refractive index was then converted to density.

Table 1. Characteristics of chum salmon specimens

| Stage              | Sex    | Age | Date and place of collection                     | Fork length (cm) | Body weight (g) | Gonadosomatic index* | Hepatosomatic index** |
|--------------------|--------|-----|--|------------------|-----------------|----------------------|-----------------------|
| Upstream migration | Male   | 04  | Nov. 15, 1985                                    | 83               | 6640            | 3.61                 | 1.78                  |
|                    | Female | 04  | Lower reaches (1.8km) of Yurappu River, Hokkaido | 82               | 5720            | 19.06                | 1.29                  |
| Spent              | Male   | 04  | Nov. 19, 1985                                    | 75               | 3920            | 0.74                 | 1.22                  |
|                    | Female | 03  | Lower reaches (1.8km) of Yurappu River, Hokkaido | 76               | 3830            | 1.83                 | 1.25                  |

\* (Gonad weight/Body weight)  $\times 100$ .

\*\* (Liver weight/Body weight)  $\times 100$ .

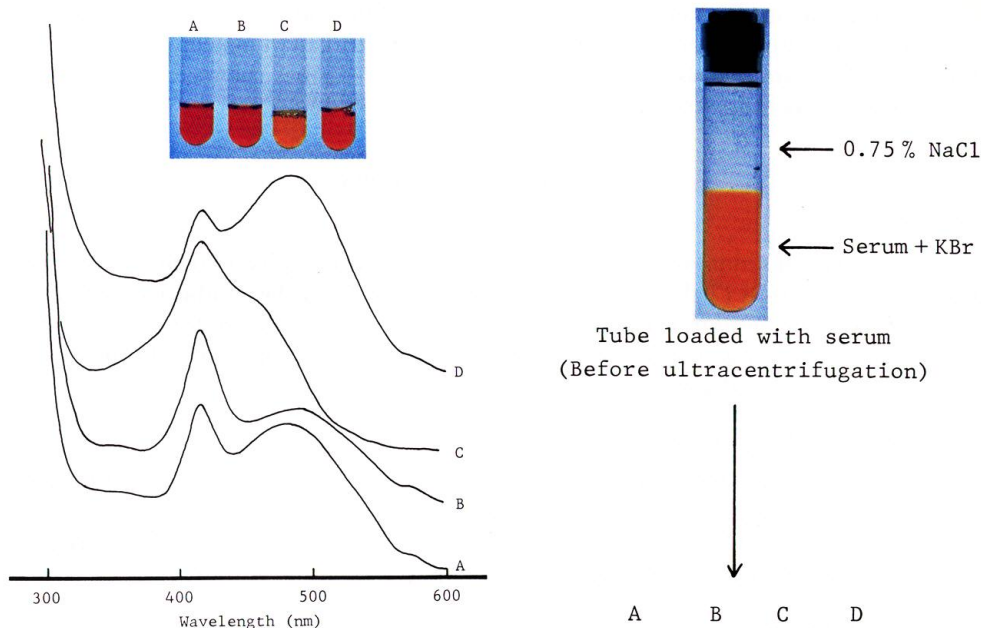
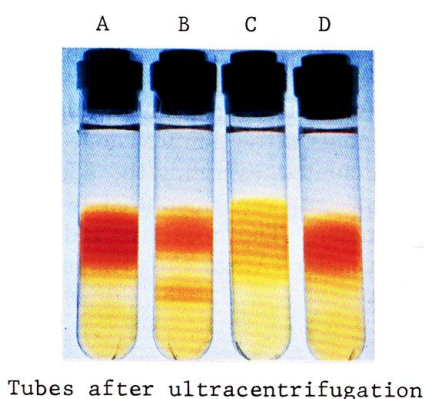


Fig. 1. Coloration and absorption spectra of serum from chum salmon: A-upstream migrating male fish; B-upstream migrating female fish; C-spent male fish; D-spent female fish.

Fig. 2. Separation of serum lipoproteins by ultracentrifugation: A-upstream migrating male fish; B-upstream migrating female fish; C-spent male fish; D-spent female fish.



### *Thin-layer chromatography (TLC) of carotenoids*

The concentrated carotenoids were examined by TLC. The high performance silica gel plate (a ready-made plate from Whatman) was developed by *n*-hexane-acetone (4 : 1, v/v).

## Results and Discussion

### *Serum coloration and absorption spectra*

The serum became bright orange or yellow during spawning migration, suggesting the presence of carotenoids (Fig. 1 and Table 2). The spectra of upstream migrating fish had two absorption maxima, one at 415 nm and the other at 480 nm. The spectrum of spent male fish had two absorption maxima, one at 420 nm and the other at 455-460 nm, although the spectrum of spent female was the same as that of

Table 2. Carotenoids level of chum salmon serum

| Stage              | Sex    | Carotenoids<br>( $\mu\text{g/ml}$ serum) |
|--------------------|--------|--|
| Upstream migration | Male   | 11.10                                    |
|                    | Female | 8.26                                     |
| Spent              | Male   | 6.35                                     |
|                    | Female | 8.14                                     |

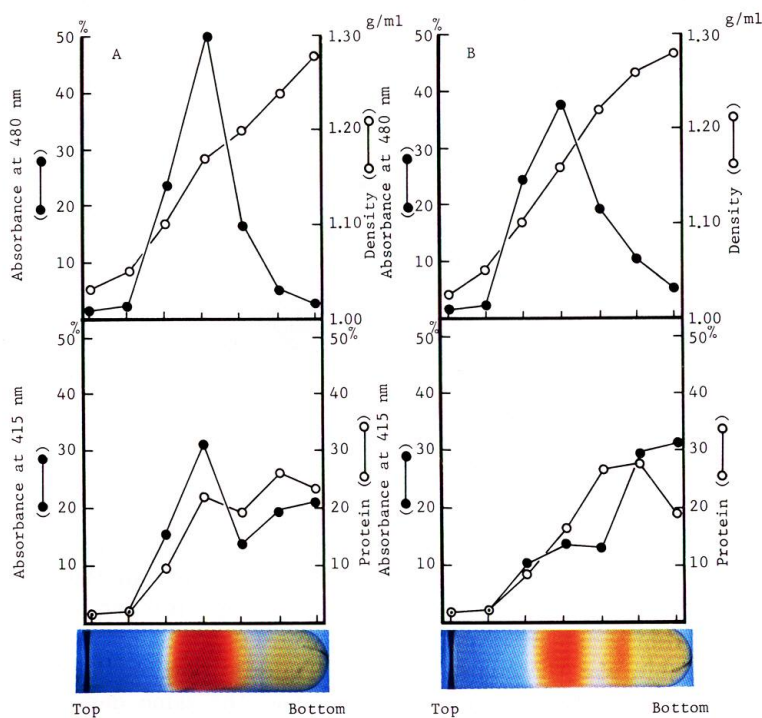


Fig. 3. Distributions of density, absorbances at 480 nm and 415 nm, and proteins in the fractions from the upstream migrating male (A) and female (B) serum after ultracentrifugation at  $218,000 \times g$  for 5 hr.

upstream migrating fish. The absorption at 415 nm and at 455–480 nm might be derived from transferrin<sup>22)</sup> or hemolysis<sup>13,14)</sup> and from carotenoids,<sup>17,18)</sup> respectively. Carotenoids composition of spent male serum might differ from those of the other fish since the absorption of carotenoids in spent male was found at 455–460 nm.

*Presence of carotenoids-carrying lipoprotein in serum*

If carotenoids are not associated with serum proteins, then by ultracentrifugation, the carotenoids can be found at the top of the tube. Density gradient ultracentrifugal analysis on all the sera confirmed that the carotenoids were associated with lipoproteins (Fig. 2). Two bands of orange were found in the middle and

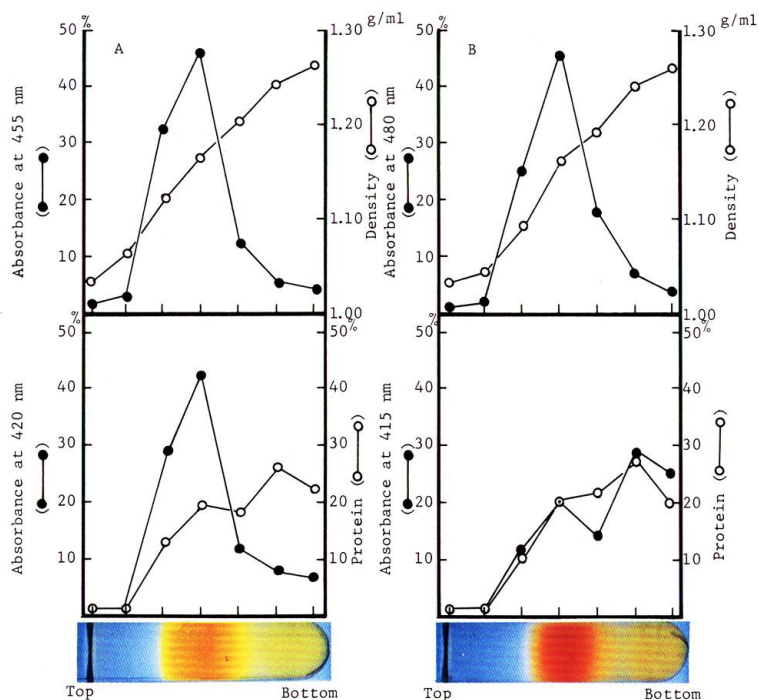


Fig. 4. Distributions of density, absorbances at 480 nm, 455 nm, 420 nm and 415 nm, and proteins in the fractions of the spent male (A) and female (B) serum after ultracentrifugation at  $218,000 \times g$  for 5 hr.

lower middle portions of the upstream migrating female serum, while only one band was found in the middle portion of the others. The band in the lower middle portion ( $d=1.22$  g/ml) might be derived from vitellogenin<sup>16)</sup> or other very high density lipoprotein.<sup>17)</sup>

More noticeable was the coloration in the middle portion of the spent male serum. The middle portion from all the sera had the same density (1.16 g/ml) corresponding to a high density lipoprotein (HDL) fraction, although the coloration in the middle portion of the spent male serum was yellowish (Figs. 3 and 4). As reported in a previous paper,<sup>17)</sup> carotenoids-carrying lipoprotein, by which muscle carotenoids (mainly astaxanthin) were transported into the integument, was isolated from the HDL fraction of upstream migrating male serum. This might suggest that carotenoids-carrying lipoprotein from the HDL fraction could transport not only astaxanthin but also yellowish carotenoids.

As shown in Figs. 3 and 4, the distribution of protein was similar to that of absorption at 415 nm. The distribution of absorption at 420 nm in the spent male serum was similar to that at 455 nm, suggesting the presence of carotenoids other than astaxanthin. The absorption at 415 nm was derived from the serum proteins, while that at 420, 455-460 and 480 nm was derived from the serum coloration. This was supported from the absorption spectra of HDL fraction and the bottom portion (Fig. 5).

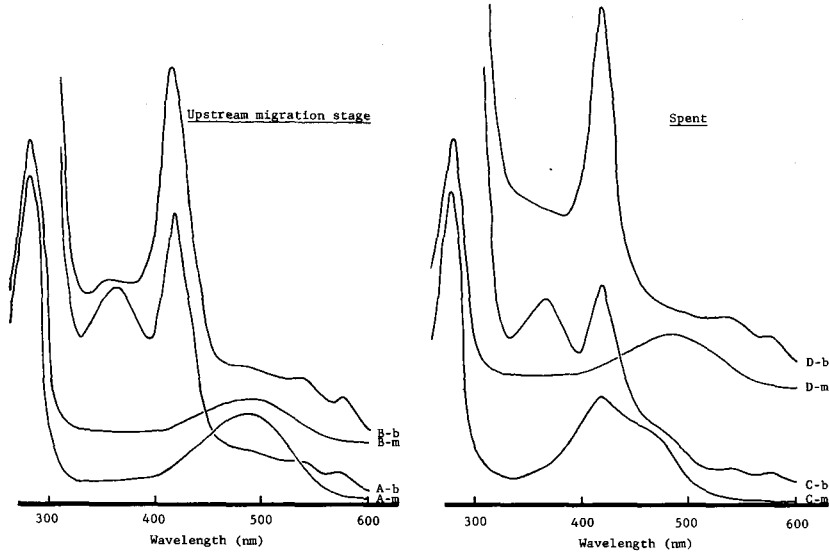


Fig. 5. Absorption spectra of serum fractions from the middle (m) and bottom (b) portions: A-upstream migrating male fish; B-upstream migrating female fish; C-spent male fish; D-spent female fish.

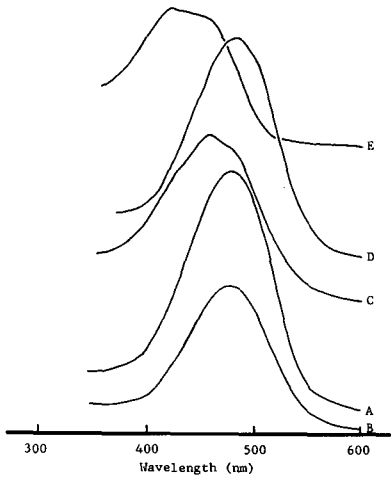


Fig. 6. Absorption spectra of carotenoids (A, B, C, D; solvent-ethanol) and water soluble yellowish pigments (E; solvent-water) from high density lipoprotein fractions: A-upstream migrating male fish; B-upstream migrating female fish; C and E-spent male fish; D-spent female fish.

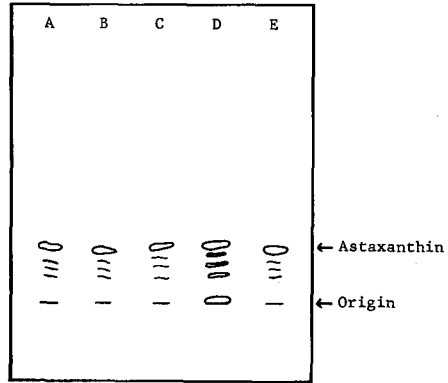


Fig. 7. High performance thin-layer chromatography of carotenoids from high density lipoprotein (A, B, D, E) and very high density lipoprotein (C) fractions: A-upstream migrating male fish; B and C-upstream migrating female fish; D-spent male fish; E-spent female fish.

### *Carotenoids of HDL fraction*

Figure 6 shows the absorption spectra of carotenoids extracted from the HDL fraction with ethanol-diethyl ether. Each spectrum, except for the spent male fish, all had absorption maxima at 480 nm, while the spectrum of the spent male had three absorption maxima; 420, 455 and 475 nm. Yellowish pigments were not completely extracted from the HDL fraction of spent male with ethanol-diethyl ether. These pigments slightly dissolved in ethanol but mostly in water. The spectrum of yellowish pigments extracted with water had absorption maximum at 420 nm (Fig. 6). This might indicate that the absorption at 455 and 475 nm was derived from carotenoids.

Carotenoids composition of the HDL fraction was studied by high performance TLC (Fig. 7). Astaxanthin was the main carotenoid in the HDL fraction except for the spent male serum, where three carotenoids, other than astaxanthin, were clearly detected. Carotenoids other than astaxanthin, however, were faintly present in the HDL fractions except for the spent male. More noticeable was the presence of an immobile band with yellowish coloration in the spent male. This band might correspond to the water soluble pigments with the absorption maxima at 420 nm. Thus, the yellowish serum found in the spent male was considered to be derived from both the difference in carotenoids composition and the presence of water soluble yellowish pigments.

Kōchiyama *et al.*<sup>23)</sup> have reported that the serum of most vertebrates is yellowish, due to the presence of small amounts of bilirubin or carotenoids. The serum bilirubin level is known for rainbow trout,<sup>24,25)</sup> coho salmon,<sup>26)</sup> carp<sup>25)</sup> and eel.<sup>27)</sup> Kawatsu and Sakai<sup>27)</sup> have found hyperbilirubinemia in cultured eel whose abdomen and fins became yellowish. If the water soluble yellowish pigments found in the spent male serum are bilirubin, the spent male fish may be in a pathological state. These results might suggest that carotenoids-carrying lipoprotein from the HDL fraction could transport bilirubin as well as carotenoids other than astaxanthin. Thus, carotenoids-carrying lipoprotein is of interest, not only from a biochemical point of view but also, from a physiological viewpoint. Further studies on detailed analyses of carotenoids composition and water soluble yellowish pigments in carotenoids-carrying lipoprotein from HDL fractions are now in progress, the results of which will be described elsewhere.

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