産卵回帰シロザケのブナ化にともなう血中各種イオンおよびホルモン濃度の変化

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Changes in Plasma Electrolyte and Hormone Concentrations during Homing Migration of Chum Salmon *Oncorhynchus keta* with Special Reference to the Development of Nuptial Color

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Returning chum salmon *Oncorhynchus keta* captured by set-nets placed along the Shiretoko Peninsula Hokkaido contain both silvery and nuptial colored fish. The nuptial color further develops in spawning fish in the river. To clarify physiological changes during nuptial coloration, plasma osmolality, electrolite concentrations, and plasma levels of testosterone, estradiol-17β, growth hormone, thyroxine, and the thickness of the skin were investigated using the fish captured in September and October. Plasma osmolality and concentrations of sodium and chloride significantly decreased in the spawning group but there was no significant difference between the silvery and the nuptial colored groups. Plasma calcium and magnesium significantly decreased during nuptial coloration in both sexes. The thickness of the skin, especially the epidermis, closely correlated with the development of nuptial color. Plasma testosterone level also increased during nuptial coloration in both sexes, but the level of females captured in the sea was much lower than that of corresponding males. Plasma concentration of thyroxine of females captured in the sea was significantly lower than that of males. Growth hormone levels increased during nuptial coloration in both sexes, and stayed high even in the river. These results indicate that the nuptial colored salmon captured in the sea have the same adapting ability to the seawater as the silvery fish, and that testosterone might promote the nuptial coloration and thickening of the skin. Sexual differences of the testosterone levels detected in the seawater salmon suggest that other hormones, such as thyroxine, may act as an antagonist to testosterone.

Chum salmon *Oncorhynchus keta* captured by set-nets on their homing (spawning) migration have been one of the most commercially important fishes of coastal fisheries in Japan. During the migration, however, secondary sexual characteristics such as nuptial color or watermark develop in both sexes and at the same time, their fresh quality decreases.[1] The development of nuptial color has been, then, experientially used by local fishermen as one of criteria to evaluate quality of salmon by their appearance.

High levels of plasma androgens during the late period of this migration of chum salmon has been reported[2] and administration of androgens promoted nuptial coloration in some salmonids.[3,4] However the precise physiological mechanisms of the development of nuptial coloration during final maturation of salmons are uncertain yet. The aim of the present study was to obtain basic data on changes of plasma concentration of some ions and hormones during nuptial coloration of chum salmon.

Materials and Methods

Fish

Chum salmon were captured by salmon set-nets placed off shore of the western coast of the Shiretoko Peninsula, Utoro, Hokkaido, in September and October, 1987 and 1988. They were transported to a tank containing seawater on land, then were anesthetized with about 0.02% tricaine methansulfonate (TMS; Crescent Research Chemicals, Arizona). They were individually labeled and body weight and folk length were recorded. Blood samples were taken from the caudal vessels with hepalinized syringes. Plasma samples were frozen on dry ice and stored at -80°C for measurements. Fish were then dissected and gonad weight was measured and gonadosomatic indices (GSI, gonad weight $\times 100$/body weight) were calculated.

For histological analysis, a portion of the skin just below the front end of the dorsal fin was removed and fixed in Bouin's solution. The skin was dehydrated and embedded in paraffin. Then serial sections (5 μm in thickness) were made and stained

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with Mayer's hematoxyline and eosin for microscopic observation. Fifty points per a specimen were randomly chosen to measure the thickness of epidermis and dermis.

The body color was divided into four classes by external appearance; silvery colored (−), faintly (±), moderately (+), and extensively nuptial colored (++). Groups of + and ++ were pooled and refered as the nuptial colored group.

To evaluate another secondary sexual characteristic, the snout and the head length were measured to the nearest millimeter and the snout-head ratio (snout length/head length) was calculated.

For comparison, some males and females were captured at the Shari River or the Iwaobetsu River at each sampling time and were processed in the same way as the fish captured in the sea. They were referred as the spawning group.

Ion and Osmorality Analysis

Plasma concentrations of sodium, calcium, and magnesium were measured by emission spectrophotometry using an atomic absorption-emission spectrophotometer (Shimazu, A610). Plasma chloride was analyzed using a chloride counter (Hiranuma, CC-1). Plasma osmorality was measured with a osmometer (Vogel, OM-801).

Hormone and Protein Analysis

Plasma concentrations of testosterone,[5] estradio-17β,[6] and growth hormone[7] were measured by specific radioimmunoassays. Plasma thyroxine levels were determined using an enzyme immunoassay kit (Enzymun-test T4, Boehringer-Mannheim-Yamanouchi, Tokyo). Plasma protein was measured using Lowry method.[8]

Values of each group were expressed as means ± SEM and were statistically analyzed using analysis of variance.

Results

Development of Nuptial Color

The lateral body color of chum salmon of silvery group was silverwhite, and their scales easily came off. As the development of nuptial color, dark brownish irregular patterns (water marks), which first appeared in the area below the lateral line, spread whole the body surface in both sexes. Spawning salmon captured in the river had a much darker body color than that of nuptial colored group. In addition to the dark brownish patterns, spawning salmon, especially males, developed drab reddish patterns.

Another secondary sexual characteristic occurred in both sexes accompanying the development of nuptial color, though it was more remarkable in males than in females (Fig. 1). In males, the snout/head ratio of the spawning and the nuptial colored groups was significantly greater than that of the silvery group ($P<0.01$, $P<0.05$, respectively). The ratio of the spawning group was also significantly greater ($P<0.05$) than that of silvery females.

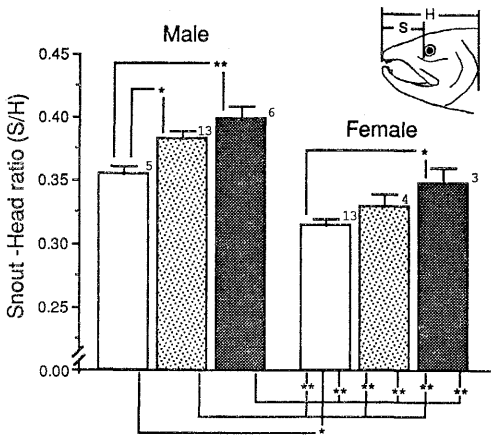

Fig. 1. Changes in snout/head ratio (snout length/head length) during nuptial coloration of male and female chum salmon. Open column, silvery group; dotted column, nuptial colored group; solid column, spawning group. *, $P<0.05$; **, $P<0.01$. Numbers near each column represent numbers of fish examined.

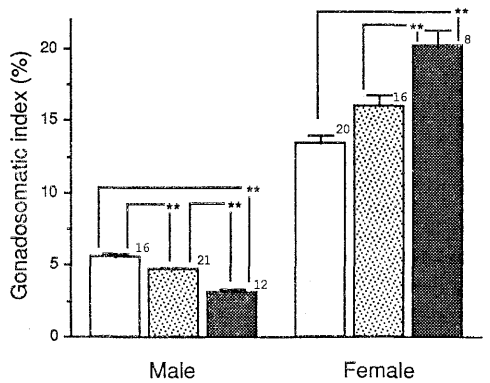

Fig. 2. Changes in gonadosomatic indices (gonad weight ×100/body weight) during nuptial coloration of male and female chum salmon. Open column, silvery group; dotted column, nuptial colored group; solid column, spawning group. **, $P<0.01$. Numbers near each column represent numbers of fish examined.

GSIs

Male GSI significantly decreased as the nuptial color developed ($P<0.01$, Fig. 2). Spermiation had occurred in some males of the nuptial colored group, and in all the males of the spawning group. In contrast female GSI increased accompanying the nuptial color development (Fig. 2). Female GSI of the spawning group was significantly greater than that of other groups ($P<0.05$).

Osmolality

In both sexes plasma osmolality of the salmon captured in the river (about 300 mOsm/kg) was significantly lower than that of the fish caught in the sea (about 400 mOsm/kg, $P<0.05$, Fig. 3). There was no significant difference between the silvery and the nuptial colored groups (Fig. 3).

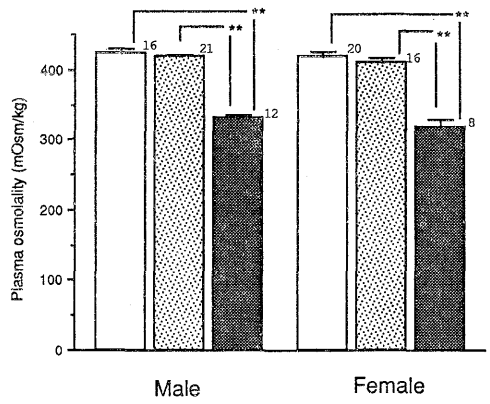

Fig. 3. Changes in plasma osmolality during nuptial coloration of male and female chum salmon. Open column, silvery group; dotted column, nuptial colored group; solid column, spawning group. **, $P<0.01$. Numbers near each column represent numbers of fish examined.

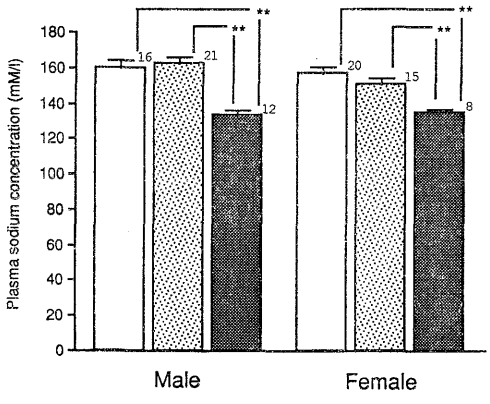

Fig. 4. Changes in plasma sodium concentrations during unptial coloration of male and female chum salmon. Open column, silvery group; dotted column, nuptial colored group; solid column, spawning group. **, $P<0.01$. Numbers near each column represent numbers of fish examined.

Sodium and Chloride

The patterns of plasma concentrations of sodium and chloride in three groups were almost the same as those of plasma osmolality (Figs. 4 and 5); concentrations of these ions of spawning

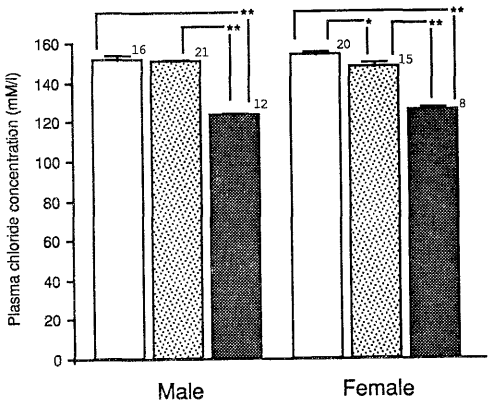

Fig. 5. Changes in plasma chloride concentrations during nuptial coloration of male and female chum salmon. Open column, silvery group; dotted column, nuptial colored group; solid column, spawning group. *, $P<0.05$; **, $P<0.01$. Numbers near each column represent numbers of fish examined.

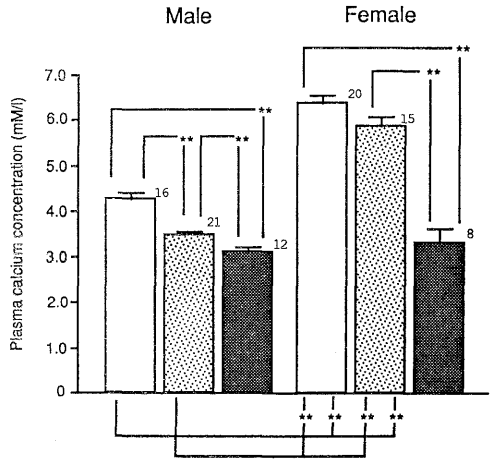

Fig. 6. Changes in plasma calcium concentrations during nuptial coloration of male and female chum salmon. Open column, silvery group; dotted column, nuptial colored group; solid column, spawning group. **, $P<0.01$. Numbers near each column represent numbers of fish examined.

(fresh-water) salmon were significantly lower than those of other two groups (sea-water, $P<0.01$). However, the concentration of plasma chloride of nuptial colored females was significantly lower than that of the silvery group (Fig. 5).

Calcium

Plasma calcium concentration in males significantly decreased as the nuptial coloration developed ($P<0.01$, Fig. 6). This tendency was also true in females, although the difference between the silvery and the nuptial colored groups was not significant (Fig. 6). In the sea-water salmon, plasma calcium concentration was significantly higher in females than in males ($P<0.01$, Fig. 6).

Magnesium

Even in the seawater the concentration of plasma magnesium clearly decreased as the nuptial color developed in both sexes ($P<0.01$, Fig. 7). It furthermore decreased in the freshwater in both sexes ($P<0.01$, Fig. 7).

Protein

Plasma protein concentration of the spawning salmon was significantly lower than that of the silvery or the nuptial colored salmon in both sexes ($P<0.05$, $P<0.01$, respectively; Fig. 8).

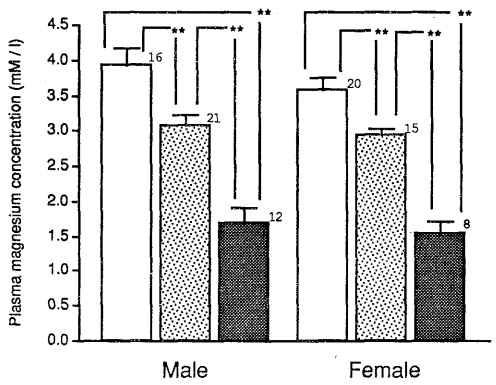

Fig. 7. Changes in plasma magnesium concentrations during nuptial coloration of male and female chum salmon. Open column, silvery group; dotted column, nuptial colored group; soid column, spawning group. **, $P<0.01$. Numbers near eac cohlumn represent numbers of fish examined.

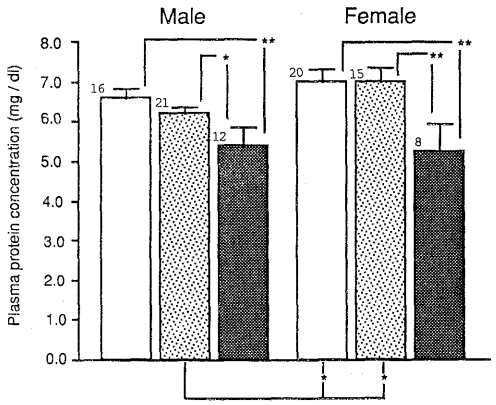

Fig. 8. Changes in plasma protein concentrations during nuptial coloration of male and female chum salmon. Open column, silvery group; dotted column, nuptial colored group; solid column, spawning group. *, $P<0.05$; **, $P<0.01$. Numbers near each column represent numbers of fish examined.

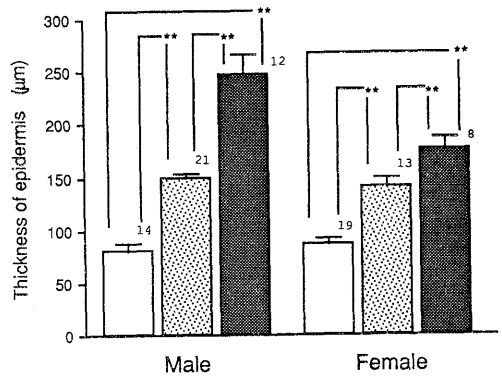

Fig. 9. Changes in thickness of epidermis during nuptial coloration of male and female chum salmon. Open column, silvery group; dotted column, nuptial colored group; solid column, spawning group. **, $P<0.01$. Numbers near each column represent numbers of fish examined.

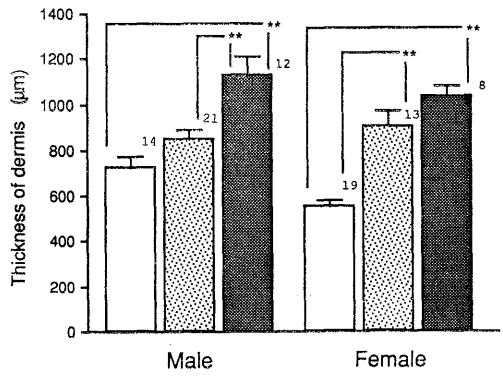

Fig. 10. Changes in thickness of dermis during nutial coloration of male and female chum salmon. Open column, silvery group; dotted column, nuptial colored group; solid column, spawning group, **, $P<0.01$. Numbers near each column represent numbers of fish examined.

The concentration of nuptial colored males was significantly lower than that of silvery or nuptial colored females ($P<0.05$, Fig. 8).

Thickness of Epidermis and Dermis

Histological observation revealed that the thickness of epidermis significantly increased during the nuptial coloration ($P<0.01$. Fig. 9). The same tendency was also detected in the dermis although differences were only partly significant (Fig. 10).

Testosterone

The concentration of plasma testosterone of male salmon increased accompanying the development of nuptial color; from about 25 ng/m*l* in the silvery group to about 40 ng/m*l* in the spawning group. The statistic significance was, however, only detected between the spawning group and other groups ($P<0.01$, Fig. 11). Almost the same pattern of variation was shown in females but the levels of female testosterone were much lower than those of males; about one fourth in the silvery and the nuptial colored groups, and about a half in the spawning group ($P<0.01$, Fig. 11).

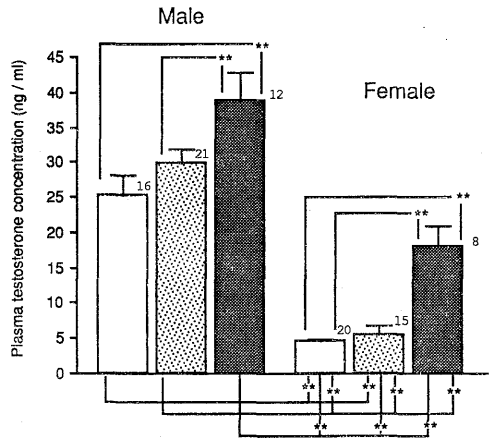

Fig. 11. Changes in plasma testosterone concentrations during nuptial coloration of male and female chum salmon. Open column, silvery group; dotted column, nuptial colored group; solid column, spawning group. **, $P<0.01$. Numbers near each column represent numbers of fish examined.

Estradiol-17β

Concentrations of plasma estradiol-17β of males was low (about 0.17 ng/m*l*) in the silvery group, then increased to about 0.3 ng/m*l* in the nuptial colored group, and significantly decreased in the spawning group ($P<0.01$, Fig. 12-A). Plasma estradiol-17β levels of female salmon gradualy decreased from about 15 to 12 ng/m*l* during the nuptial coloration in the sea, then drastically decreased to about one ng/m*l* in the spawning group ($P<0.01$, Fig. 12-B).

Growth Hormone

Plasma GH was measured only for the fish captured in 1987. In male salmon, the concentration of plasma GH increased during nuptial coloration; about 8 ng/m*l* in the silvery group to about 22 ng/m*l* in the spawning group (Fig. 13). The GH level of spawning males was significantly higher compared to that of the silvery group ($P<0.05$) or the nuptial colored group ($P<0.01$). In females plasma GH had already significantly increased from about 9 ng/m*l* in the silvery group to about

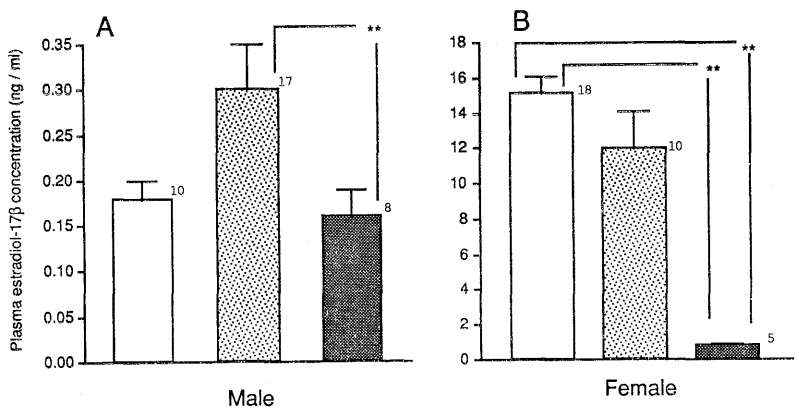

Fig. 12. Changes in plasma estradiol-17β concentrations during nuptial coloration of chum salmon. A, males; B, females. Open column, silvery group; dotted column, nuptial colored group; solid column, spawning group. **, $P<0.01$. Numbers near each column represent numbers of fish examined.

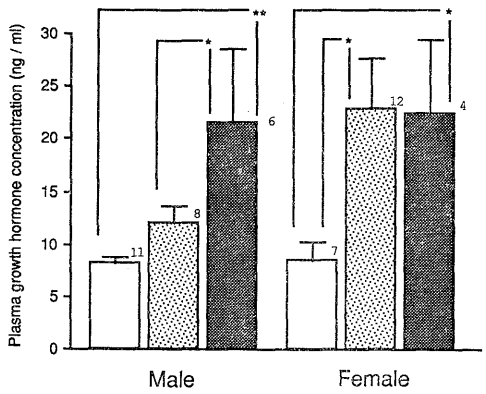

Fig. 13. Changes in plasma growth hormone concentrations during nuptial coloration of male and female chum salmon. Open column, silvery group; dotted column, nuptial colored group; solid column, spawning group. *, $P<0.05$; **, $P<0.01$. Numbers near each column represent numbers of fish examined.

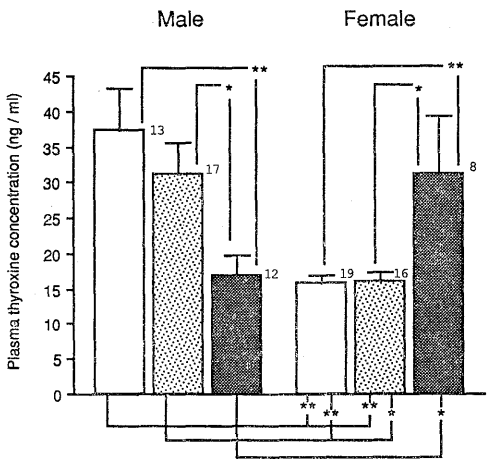

Fig. 14. Changes in plasma thyroxine concentrations during nuptial coloration of male and female chum salmon. Open column, silvery group; dotted column, nuptial colored group; solid column, spawning group. *, $P<0.05$; **, $P<0.01$. Numbers near each column represent numbers of fish examined.

22 ng/m*l* in the nuptial colored group ($P<0.05$, Fig. 13). This GH level was maintained in the spawning females though the differences between individuals were great (Fig. 13).

Thyroxine

In contrast to GH, concentrations of plasma thyroxine of males decreased during nuptial coloration from about 37 ng/m*l* in the silvery group to about 17 ng/m*l* in the spawning group with significant differences between the silvery and the spawning groups ($P<0.01$), and between the nuptial colored and the spawning groups ($P<0.05$, Fig. 14). Being different from that of males, the thyroxine level of spawning females (about 30 ng/m*l*) was significantly higher than that of the silvery or the nuptial colored group (about 15 ng/m*l* each, $P<0.01$, $P<0.05$, respectively, Fig. 14). Moreover, the thyroxine levels of silvery or nuptial colored males were significantly higher than those of silvery or nuptial colored females ($P<0.01$–0.05, Fig. 14).

Discussion

Changes of GSI values represented the proceeding of maturation during nuptial coloration of chum salmon; in males, the number of spermiated fish increased accompanying the nuptial coloration and the decrease of GSI was due to the loss of sperms when the testes were dissected. In females, yolk accumulation was almost completed in both the silvery and the nuptial colored groups; the significant increase of GSI in the spawning group seems to be due to the hydration of the overies because all the females of the group had ovulated.

The changes of plasma osmolality and concentrations of sodium and chloride ions represented almost the same pattern; the fish captured in the freshwater had significantly lower values than those of the fish captured in the seawater, and there was no significant difference between the silvery and the nuptial colored groups except the chloride concentration of females (Figs. 3–5). These results indicate that the plasma osmolality which is mainly caused by sodium and chloride ions is affected by environmental water, and that the nuptial colored group have yet almost the same adapting ability to the seawater as that of the silvery group. On the other hand, spawning salmon captured in the river are reported to have lost the ability.[9]

Concentrations of calcium and magnesium ions significantly decreased during nuptial coloration (Figs. 6 and 7). Since these ions are regulating factors of some enzymes, such changes of concentration may reflect the changes of basic metabolism during nuptial coloration or maturation. In addition, magnesium is reported to accumulate in the ovary accompanying the advancement of maturation in rainbow trout,[10] but it is uncertain whether such is the case in the testis. Significantly higher concentrations of female plasma calcium of the

silvery and the nuptial colored groups than those of males seem to be due to the production of yolk material which is glycolipophosphoprotein containing calcium.[11] Higher levels of female plasma protein of sea-water salmon also seem to be due to the circulating yolk material (Fig. 8).

Thickness of the skin, especially epidermis, significantly increased during nuptial coloration (Figs. 9 and 10). This phenomenon is thought to be a kind of protective adaptation for the spawning in the river, and external administrations of androgens could cause this change, as well as the induction of darkener of body color.[12-14] In this study, testosterone levels also increased during nuptial coloration, though we failed to detect significant differences between the silvery and the nuptial colored groups in both sexes (Fig. 11). However, plasma concentrations of testosterone of female salmon captured in the sea were significantly lower than those of males; the testosterone level of nuptial colored females was only about one fifth of that of silvery males (Fig. 11). These results suggest that some factors other than androgens affect the nuptial coloration or the thickness of the skin in chum salmon. One of the possible factors is plasma thyroxine which promote silvering of body color of salmonids during smoltification.[13,15] In this study we have revealed that the plasma thyroxine levels of male salmon captured in the sea were significantly higher than those of corresponding females (Fig. 14). The higher levels of thyroxine, then, may have an antagonistic effect to androgens in inducing nuptial coloration or thickening of the skin.

Plasma growth hormone levels increased during nuptial coloration in the sea in both sexes, especially in female. This result coincides with that of Hirano *et al.*[9] who kept the maturing chum salmon in the seawater for several days. The increase of plasma GH in the seawater salmon seems to have acted to compensate for the decrease of the ability to adapt to the seawater, and as the result, plasma osmolality may not have changed. High levels of GH in the freshwater salmon, however, is unaccountable. It is required to clarify whether these high levels of GH have a physiological role or not.

Acknowledgements

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