

## Changes in Chum Salmon Plasma Levels of Steroid Hormones during Onset of the Spawning Migration in the Bering Sea

Takeshi Onuma<sup>1</sup>, Shunpei Sato<sup>1,2</sup>, Aya Jodo<sup>1</sup>, Nancy D. Davis<sup>3</sup>, Hironori Ando<sup>1</sup>,  
Masatoshi Ban<sup>2</sup>, Masa-aki Fukuwaka<sup>4</sup>, and Akihisa Urano<sup>1,5</sup>

<sup>1</sup>Division of Biological Sciences, Graduate School of Science, Hokkaido University,  
Sapporo, Hokkaido 060-0810, Japan

<sup>2</sup>National Salmon Resources Center,

2-2 Nakanoshima, Toyohira-ku, Sapporo, Hokkaido 062-0922, Japan

<sup>3</sup>School of Aquatic and Fishery Sciences, University of Washington  
Box 355020, Seattle, WA 98195-5020, USA

<sup>4</sup>Hokkaido National Fisheries Research Institute, Fisheries Research Agency,  
116 Katsurakoi, Kushiro, Hokkaido 085-0802, Japan

<sup>5</sup>Field Science Center for Northern Biosphere, Hokkaido University,  
Sapporo, Hokkaido, 060-0809 Japan



Keywords: Migration, chum salmon, Bering Sea, maturation, testosterone, 11-ketotestosterone, estradiol-17 $\beta$ , HPG-axis

After a few years of oceanic life, Japanese chum salmon migrate to their natal rivers from the Bering Sea. Previous studies have demonstrated involvement of the brain-pituitary-gonadal (HPG) axis in regulation of the spawning migration. In homing chum salmon, expression of genes encoding salmon gonadotropin-releasing hormone (GnRH) was elevated in the forebrain during upstream migration from the coast to the natal hatchery (Onuma et al. 2004, in press). In the pituitary, amounts of mRNA encoding gonadotropin (GTH) II $\beta$  and somatolactin were elevated in stocks migrating a long river (Kitahashi et al. 1998; Taniyama et al. 1999) and a short river upstream (Onuma et al. 2003b). Plasma levels of testosterone (T), 11-ketotestosterone (11KT) and estradiol-17 $\beta$  (E2) decreased along with final gonadal maturation, while those of 17 $\alpha$ -20 $\beta$ -dihydroxy-4-pregnen-3-one (DHP) and cortisol elevated at the final phases of the spawning migration (Onuma et al. 2003a, b).

Plasma levels of sex steroid hormones of chum salmon in the North Pacific Ocean were lower when compared to those of pre-spawning fish (Ueda et al., 1984; Ueda 1998). In farmed masu salmon, plasma levels of T, 11KT and E2 elevated with gonadal maturation from June through August (Munakata et al. 2001; Kitahashi et al. 2004). These results suggest involvement of the HPG-axis in initiation of the spawning migration in the Bering Sea. However, previous studies regarded all offshore fish as one sexually immature group (Ueda et al. 1984; Ueda 1998), which was insufficient to assess the neuroendocrine events associated with onset of the spawning migration. Therefore, in this study we examined changes in chum salmon plasma levels of steroid hormones in fish of several ages in the Bering Sea.

Chum salmon in the Bering Sea were sampled along the 180°-longitude line from late-June through mid-July 2001–2003 during the cruise of research vessel (RV) *Wakatake maru*. In September 2002 and 2003, fish were sampled during the cruise of RV *Kaiyo maru*. In addition, pre-spawning fish were sampled at seven locations along the homing pathway to their natal hatchery in Hokkaido, from mid-September through early-October 2001–2003. Blood samples were collected from the caudal vasculature, kept on ice, and centrifuged to obtain plasma. Gonadal maturity was assessed using gonadosomatic indices (GSI, gonad weight/body weight x 100). Fish age was determined by examination of scale patterns. Plasma levels of T, 11-KT, E2 and DHP were determined by enzyme immunoassays. Mitochondrial DNA haplotypes of genealogical clades were determined to distinguish between clade A (Japanese population) and clade B (Japanese, Russian and North American populations) by the recently developed DNA microarray hybridization method (Moriya et al. in press).

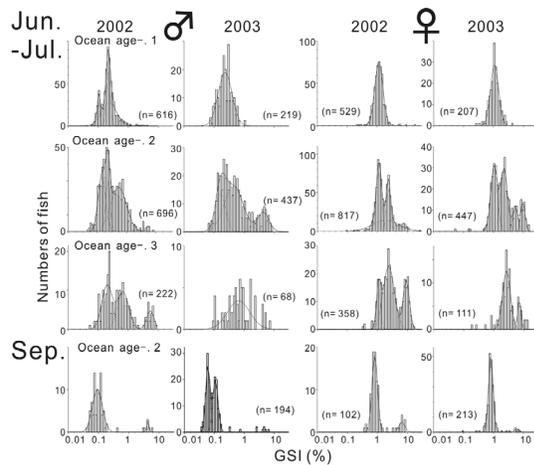
On the basis of GSI, most ocean age -.1 chum salmon in the Bering Sea were immature from late-June through mid-July, while ocean age -.2 and -.3 fish comprised several populations of immature and maturing fish (Fig. 1). In September, almost all fish were immature regardless of the age. These results indicated that most maturing fish already departed the survey areas for their natal river by the end of summer.

Plasma levels of T, 11KT and E2 were positively correlated with GSI in both sexes (Fig. 2). The levels of T and 11KT in immature male chum salmon were about 1 nM, while those of maturing fish were more than 10 nM. The levels of T and E2 in female fish showed a 10- to 50-fold elevation coincident with gonadal maturation. The levels of most ocean age -.1 fish were less than 1 nM, and in ocean age -.2 fish, the levels were variable among individuals. The levels of T and E2 in ocean age -.3 female fish were more than 10 nM. In contrast, DHP levels showed no correlation with GSI.

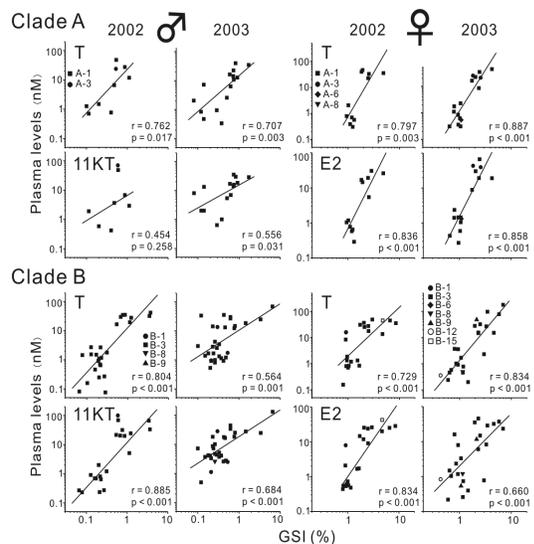
Mitochondrial DNA haplotypes were determined to examine whether the increase in plasma levels of sex steroid hormones could be observed in the Japanese population. Levels of T, 11KT, and E2 were elevated in genealogical clade A (Japanese population) and clade B (mixture of Japanese, Russian and North American populations) (Fig. 3). The elevated levels were 10–100 nM in both clades, although the maximum GSI value in clade B was higher than those in clade A.

Plasma sex steroid hormone levels in maturing chum salmon in the Bering Sea (male, GSI > 1.0; female, GSI > 2.0) were compared with the levels of pre-spawning fish during their upstream migration (Fig. 4). Elevated T and 11KT levels in male maturing fish in the Bering Sea were similar to those in pre-spawning fish captured at the coast of Hokkaido. Levels of T and E2 showed 2- to 10-fold elevation in female maturing fish until they approached the coast. Afterward, the levels increased in both sexes until fish had reached midway up their natal river, and then levels decreased during further upstream migration to the hatchery. In contrast, levels of DHP were elevated at the natal hatchery during final gonadal maturation.

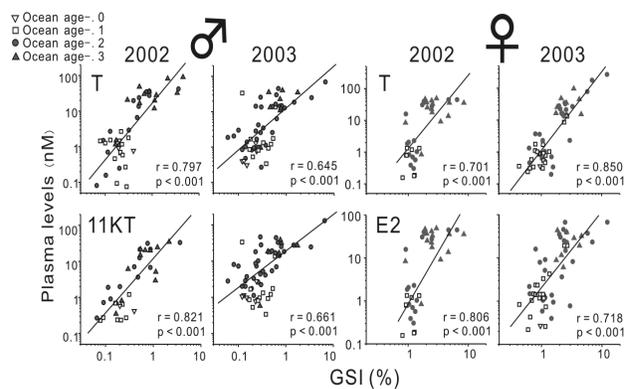
**Fig. 1.** Histograms depicting the relationship between GSI (%) and chum salmon ocean age for June–July and September, 2002–2003. The solid line combined with a few dashed lines are the fitted curves obtained by a Gaussian distribution with multiple peaks. Compare the higher GSI of maturing ocean age .2 and .3 fish with ocean age .1 fish.



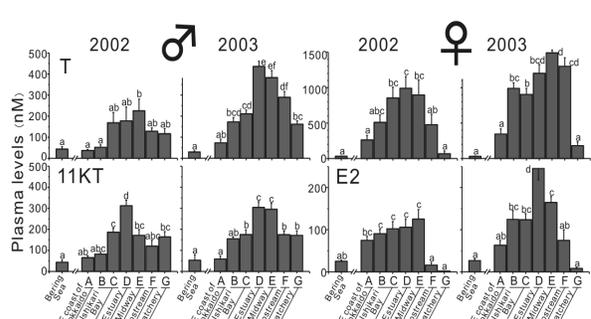
**Fig. 3.** Scatterplots showing the relationship between GSI (%) and plasma levels (nM) of steroid hormones in genealogical clade A and B. Clade A represents the Japanese population, while clade B is a mixture of Russian, North American, and Japanese populations. Elevations of T, 11KT and E2 plasma levels were observed in both clades.



**Fig. 2.** Scatterplots relating GSI (%) and plasma levels (nM) of steroid hormones in chum salmon captured in the Bering Sea. Note the levels of T, 11KT and E2 are elevated with increasing fish age and GSI.



**Fig. 4.** Histograms indicating plasma levels (nM) of sex steroid hormones in maturing fish caught in the Bering Sea (male, GSI > 1.0; female, GSI > 2.0) and along the spawning migration pathway. Each value represents the mean standard error. Significant differences identified by letter combinations ( $p < 0.05$  one-way ANOVA, Tukey test).



These results clearly indicate that levels of T, 11KT and E2 become elevated in the Bering Sea during onset of the spawning migration to the natal river, regardless of the chum salmon source population. Levels of approximately 10 nM stimulated synthesis and release of GnRH (Breton and Sambroni 1996) and GTHs (Ando *et al.* 2003, 2004) in farmed fish that were immature and in the early phases of sexual maturation. Therefore, we suggest that activation of the HPG-axis occurs in the Bering Sea when chum salmon initiate their spawning migration, as was observed from June through August in farmed masu salmon (Munakata *et al.* 2001; Kitahashi *et al.* 2004). Changes in expression of genes encoding neurohormone and pituitary hormone prior to onset of chum salmon spawning migrations are under investigation.

## REFERENCES

- Ando, H., P. Swanson, and A. Urano. 2003. Regulation of LH synthesis and release by GnRH and gonadal steroids in masu salmon. *Fish. Physiol. Biochem.* 28: 61–63.
- Ando, H., P. Swanson, T. Kitani, N. Koide, H. Okada, H. Ueda, and A. Urano. 2004. Synergistic effects of salmon gonadotropin-releasing hormone and estradiol-17 $\beta$  on gonadotropin subunit gene expression and release in masu salmon pituitary cells in vitro. *Gen. Comp. Endocrinol.* 137: 109–121.
- Breton, B., and E. Sambroni. 1996. Steroid activation of the brain-pituitary complex gonadotropic function in triploid rainbow trout *Oncorhynchus mykiss*. *Gen. Comp. Endocrinol.* 101: 155–164.
- Kitahashi, T., H. Ando, M. Ban, H. Ueda, and A. Urano. 1998. Changes in the levels of gonadotropin subunit mRNAs in the pituitary of pre-spawning chum salmon. *Zool. Sci.* 15: 753–760.
- Kitahashi, T., R.K. Bhandari, S. Taniyama, H. Ando, H. Ueda, and A. Urano. 2004. Changes in expression of salmon GnRH genes and responsiveness of pituitary hormone genes to GnRH analog during growth and sexual maturation in masu salmon. *In Proceedings of the 5th Congress of the Asia and Oceania Society for Comparative Endocrinology*. Nara. pp. 92–94.
- Moriya, S., S. Urawa, O. Suzuki, A. Urano, and H. Abe. In press. DNA microarray for rapid detection of mitochondrial DNA haplotypes of chum salmon. *Mar. Biol.* 50.
- Munakata, A., M. Amano, K. Ikuta, S. Kitamura, and K. Aida. 2001. The involvement of sex steroid hormones in downstream and upstream migratory behavior of masu salmon. *Comp. Biochem. Physiol. B* 129: 661–669.
- Onuma, T., Y. Higashi, H. Ando, M. Ban, H. Ueda, and A. Urano. 2003a. Year-to-year differences in plasma levels of steroid hormones in pre-spawning chum salmon. *Gen. Comp. Endocrinol.* 133: 199–215.
- Onuma, T., T. Kitahashi, S. Taniyama, D. Saito, H. Ando, and A. Urano. 2003b. Changes in expression of genes encoding gonadotropin subunits and growth hormone/prolactin/somatolactin family hormones during final maturation and freshwater adaptation in prespawning chum salmon. *Endocrine* 20: 23–34.
- Onuma, T., M. Higa, H. Ando, M. Ban, and A. Urano. 2004. Changes in expression of genes encoding precursors of salmon gonadotropin-releasing hormone in discrete loci of the forebrain in pre-spawning chum salmon. *In Program of the Society for Neuroscience 34th annual meeting, San Diego, CA*. Abstract 756. 18
- Onuma, T., M. Higa, H. Ando, M. Ban, and A. Urano. In press. Elevation of gene expression for salmon gonadotropin-releasing hormone in discrete brain loci of pre-spawning chum salmon during upstream migration. *J. Neurobiol.*
- Taniyama, S., T. Kitahashi, H. Ando, M. Ban, H. Ueda, and A. Urano. 1999. Changes in the levels of mRNAs for GH/PRL/SL family and Pit-1/GHF-1 in the pituitaries of pre-spawning chum salmon. *J. Mol. Endocrinol.* 23: 189–198.
- Ueda, H., O. Hiroi, A. Hara, K. Yamauchi, and Y. Nagahama. 1984. Changes in serum concentration of steroid hormones, thyroxine, and vitellogenin during spawning migration of the chum salmon, *Oncorhynchus keta*. *Gen. Comp. Endocrinol.* 53: 203–211.
- Ueda, H. 1998. Correlations between homing, migration, and reproduction of chum salmon. *N. Pac. Anadr. Fish Comm. Bull.* 1: 146–162.