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# EXPRESSION OF HORMONE GENES AND OSMOREGULATION IN HOMING CHUM SALMON: A MINIREVIEW

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**Abstract**

Pacific salmon migrate from ocean through the natal river for spawning. Information on expression of genes encoding osmoregulatory hormones and migratory behavior is important for understanding of molecular events that underlie osmoregulation of homing salmon. In the present article, regulation of gene expression for osmoregulatory hormones in pre-spawning salmon was briefly reviewed with special reference to neurohypophysial hormone, vasotocin (VT), and pituitary hormones, growth hormone (GH) and prolactin (PRL). Thereafter, we introduced recent data on migratory behavior from SW to FW environment. In pre-spawning chum salmon, the hypothalamic VT mRNA levels increased in the males, while decreased in the females with loss of salinity tolerance when they were kept in SW. The amounts of GH mRNA in the pituitary decreased during ocean migration prior to entrance into FW. Hypo-osmotic stimulation by SW-to-FW transfer did not significantly affect the amount of PRL mRNA, but it was elevated in both SW and FW environments along with progress in final maturation. Behaviorally, homing chum salmon continued vertical movement between SW and FW layers in the mouth of the natal river for about 12 hours prior to upstream migration. Pre-spawning chum salmon in an aquarium, which allowed fish free access to SW and FW, showed that individuals with the lower plasma testosterone (T) and higher estradiol-17β (E2) levels spent longer time in FW when compared with the SW fish. Taken together, neuroendocrine mechanisms that underlie salt and water homeostasis and migratory behavior from SW to FW may be under the control of the hypothalamus-pituitary -gonadal axis in pre-spawning salmon.

Key Words: salmon; vasotocin; growth hormone; prolactin; sex steroids; upstream migration

## 1 Introduction

Most salmonid species migrate from ocean through the natal river for spawning (Salo, 1991). A success in spawning migration requires transition of mechanisms that govern water and salt homeostasis during migration from seawater (SW) through fresh water (FW), including the osmoregulatory controls with various peptidergic and proteinaceous hormones, such as neurohypophysial hormone, vasotocin (VT) (Urano et al., 1994), growth hormone (GH) (Sakamoto et al., 1993), and prolactin (PRL) (Hirano et al., 1987) in combination with cortisol (McCormick, 2001).

In pre-spawning chum salmon, salt and water homeostasis, and gonadal maturation are highly influenced by year-to-year variation of oceanic environments, such as ambient water temperature and the distribution of cold current ( Saito et al., 2001; Onuma et al., 2003a). Homing behavior of pre-spawning chum salmon in the Ishikari Bay (Kitahashi et al., 2000) and the Sanriku coast (Tanaka et al., 2000) was dependent on ocean temperature, because salmon is a typical cold-water fish. Information on migratory behavior in association with environmental factors is therefore necessary to discuss the neuroendocrine events through which homing salmon adapt to FW, although there is little available information.

In the present article, we have overviewed current understanding of changes in expression of genes encoding VT, GH and PRL in pre-spawning salmon during spawning migration, and then introduced our recent data on homing behavior of pre-spawning chum salmon during upstream migration from SW through FW. These results enable us to comprehensively discuss molecular mechanisms that underlie salt and water homeostasis in homing salmon.

## 2 Expression of osmoregulatory hormone genes in pre-spawning salmon

We have accumulated data on changes in expression of VT, GH and PRL genes in pre-spawning chum salmon during upstream migration to the long Ishikari river, and the short

Otsuchi river from 1993 through 2003. In addition, effects on gene expression for these osmoregulatory hormones of hypo-osmotic stimulation by transition from SW to FW were examined in 1994 and 1995 using pre-spawning chum salmon captured in the Otsuchi coast.

**2-1 Vasotocin**

2-1-1 Changes in VT gene expression during upstream migration

In pre-spawning chum salmon, the amounts of mRNAs encoding two VT precursors in the forebrains of females similarly decreased during upstream migration to both the long Ishikari river (Hiraoka et al., 1997) and the short Otsuchi river (Hiraoka et al., 1996; Saito et al., 2001). In the females, the intensities of hybridization signals for VT mRNAs in the preoptic nucleus magnocellularis (PM) were significantly lower in the fish arrived at the natal hatchery than those at the coast (Ota et al., 1996). Expression of VT genes in female pre-spawning chum salmon thus seemed to be repressed during upstream migration from SW through FW.

2-1-2 Hypo-osmotic stimulation is not essential

In immature rainbow trout, hypo-osmotic stimulation by transition from SW to FW elevated the intensities of hybridization signals for VT mRNAs in the PM, suggesting that VT has physiological important roles in FW adaptation (Hyodo and Urano, 1991). In pre-spawning chum salmon, however, the SW-to-FW transfer did not apparently affect the amounts of VT mRNAs in the forebrains in 1993 and 1995, although the amounts decreased after transfer to FW in 1994 (Hiraoka et al., 1996). Hypo-osmotic stimulation during migration from SW through FW seemed not to be critical to modulate expression of VT genes in pre-spawning chum salmon.

2-1-3 Salinity tolerance

Transition, rather than switching over, from SW-adapted to FW-adapted homeostatic state occurs in pre-spawning salmon along with the progress in final maturation. The plasma

levels of $Na^+$ gradually elevated within a few days, when pre-spawning chum salmon in the Otsuchi Bay were retained in SW, followed by high mortality (Hirano et al., 1990; Saito et al., 2001). The amounts of VT mRNAs in the forebrains increased in the males, but decreased in the females coincidently with the loss of salinity tolerance in SW (Hiraoka et al., 1996; Saito et al., 2001). Such changes were obvious in pre-spawning chum salmon in 1994 when the warm current (Kuroshio) was dominant in the Sanriku coast and the homing fish in the Ostuch Bay completed final maturation, while not in 1995 when the cold current (Oyashio) dominated and the fish were not fully matured (Saito et al., 2001). These facts indicate that the salt and water balance in homing salmon depends on sexual maturation, which is under influence of environmental factors. With similar experiences, we concluded that, in pre-spawning chum salmon, expression of VT genes is modulated in response to salinity stress with progress in final maturation.

**2-2 GH**

2-2-1 Spawning migration

In pre-spawning chum salmon, the amounts of GH mRNA did not show significant changes during upstream migration from 1994 through 1999 (Onuma et al., 2003b; Taniyama et al., 1999). The plasma levels of GH in the fish in the Ostuchi river were not significantly different from those in the Ostuchi Bay (Kakizawa et al., 1995). These previous studies claimed that expression of GH gene was not modulated during upstream migration. However, we recently found that the amounts of GH mRNA in the pituitary of pre-spawning chum salmon at the coast were significantly lower than those in the Bering Sea, in correlation with the gill $Na^+$, $K^+$-ATPase activities (Onuma et al., in press). Such decreases in the amounts of GH mRNA were also observed during spawning migration off the coast through the Ostuchi Bay (Onuma et al., 2003b). Gene expression for GH thus seems to be repressed

during ocean migration prior to entrance into the natal river in association with the loss of salinity tolerance in pre-spawning or matured chum salmon (Hirano et al., 1990).

**2-3 PRL**

2-3-1 Upstream migration

The amounts of PRL mRNA in the pituitaries of pre-spawning chum salmon in the Ishikari river were significantly higher than those in the Ishikari Bay in 1994 and 1995 (Taniyama et al., 1999). The amounts of PRL mRNA in the Otsuchi river were also significantly higher than those in the Ostuchi Bay (Onuma et al., 2003b) regardless of the length of river. These previous reports seemed to indicate that expression of PRL gene was elevated during upstream migration from SW through FW environments, probably at the entrance to FW. However, comparison of data from 1995 through 1999 suggested that the amounts of PRL mRNA were already elevated before entrance into the FW environment in 1998 and 1999, when the warm current was dominant in the Ishikari Bay, in contrast to 1995 and 1997, when the cold current dominated.

2-3-2 SW-to-FW transfer

Ogasawara et al. (1996) reported that the turnover rate of PRL in pre-spawning chum salmon elevated within a few days after transition from SW through FW. The SW-to-FW transfer nonetheless did not significantly alter the amounts of PRL mRNA in the pituitaries of pre-spawning chum salmon in 1994 and 1995, although the plasma levels of $Na^+$ decreased within one day after FW transfer (Onuma et al., 2003b). The amounts of PRL mRNA increased within a few days in both SW and FW environments along with progress in final maturation (Onuma et al., 2003b). We thus considered that hypo-osmotic stimulation by SW-to-FW transfer is not critical to modulate expression of PRL gene in pre-spawning salmon.

2-3-3 Influence of gonadal maturation

The SW-to-FW transfer experiments using pre-spawning salmon showed that the amounts of PRL mRNA elevated even in SW environment, coincidently with decreases in the plasma levels of testosterone (T) and estradiol-17β (E2) during final maturation (Onuma et al., 2003b). We recently examined the direct effects of salmon GnRH (sGnRH) and sex steroid hormones on the primary pituitary cells from pre-spawning masu salmon, and demonstrated that treatments of the cells with T, 11-ketotestosterone and E2 modulated PRL gene expression (Onuma et al., 2005a). Sex steroid hormones may participate in the regulation of gene expression for PRL during spawning migration.

## 3 Upstream migratory behavior

There is a paucity of information concerning homing behavior from SW through FW, although homing behavior of chum salmon are examined in the Ishikari stock (Kitahashi et al., 2000) and the Otsuchi stock (Tanaka et al., 2000) by use of data loggers. We recently clarified temporal profiles of homing behavior of pre-spawning chum salmon from SW through FW environment (Makino et al., in press). The results suggested that migratory behavior from SW through FW is closely associated with the progress in gonadal maturation.

### 3-1 Upstream migratory behavior in the Ishikari

We first investigated a temporal profile of migratory behavior of pre-spawning chum salmon. Pre-spawning fish were collected at the estuary of the Ishikari River, released after attachment of a micro data logger which can record salinity, ambient water temperature and water depth, and re-captured at the hatchery in 2005. The fish continued vertical movement between SW and FW environments at the mouth of the river for about 12 hours, followed by continuous migration in FW (Fig. 1) (Makino et al., in press). In the Ostuchi stock, the

plasma levels of $Na^+$ declined during migration from the Sanriku coast through the Otsuchi Bay prior to the entrance into FW (Saito et al., 2001). Such predictive decreases in the plasma $Na^+$ levels in SW fish were observed in 1992, when pre-spawning fish in the Bay was not fully matured, however not in 1993, when the SW fish completed final maturation and faced the loss of salinity tolerance. These results suggested that homing fish wander to and fro between SW and FW areas for a while, and actively adjusted the plasma $Na^+$ levels toward the forthcoming FW environment.

### 3-2 Aquarium experiment in Otsuchi

In addition to observation of wild fish, we investigated spontaneous behavior of pre-spawning chum salmon between SW and FW environments prepared in large aquaria. Pre-spawning chum salmon in the Otsuchi Bay were reared in a large aquarium, which allows fish to migrate between separated SW and FW streams. Plasma was collected from each fish every morning, and the relationship of plasma sex steroid hormones with the ratio of time spent in FW was estimated on the basis of data obtained by use of micro data loggers. The plasma levels of T in the individuals which spent time mainly in FW were lower or declined earlier, when compared to those in the individuals which stayed mainly in SW (Fig. 2) (Makino et al., in press). The plasma E2 levels in the males tended to be lower in FW than in SW, while those in the females were initially rather higher in FW than in SW and then declined. In combination with the patterns of changes in the plasma levels of sex steroid hormones shown in Fig. 3, we considered that, in pre-spawning chum salmon, a preference to FW in homing fish seems to be related to circulating levels of sex steroid hormones.

### 3-3 Changes in the plasma levels of sex steroid hormones during upstream migration

Sex steroid hormones, such as T, 11KT and E2, facilitated upstream migratory behavior of

sockeye salmon (Kitahashi et al., 1998) and masu salmon (Munakata et al., 2001). The plasma levels of sex steroid hormones in pre-spawning chum salmon during upstream migration were thus determined from 2001 to 2005 (Fig. 3) (Makino et al., in press). The levels of T increased during upstream migration and peaked on the midway of the river, followed by decreases at the hatchery in both sexes. The levels of E2 also reached their peaks at the estuary or on the midway of the river in all years examined. We thus consider that sex steroid hormones modulate preference to FW and facilitate upstream migratory behavior from SW through FW.

### 3-4 The HPG axis and upstream migration

In salmonid species, salmon gonadotropin-releasing hormone (sGnRH) neurons are the typical neuroendocrine cells that integrate information, and regulate reproductive behavior and pituitary hormone secretion (Onuma et al., 2005b). In homing chum salmon, expression of sGnRH genes was elevated in almost all forebrain loci during upstream migration from the coast through the natal hatchery (Onuma et al., 2005b). Administration of GnRH analogue stimulated preference to FW, and also shortened homing duration in pre-spawning chum salmon from the mouth of the Ishikari River to the hatchery (Kitahashi et al., 2001). We therefore consider that migratory behavior from SW through FW environment is, in part, regulated by sGnRH neurons.

### 3-5 Concordant regulation of osmoregulatory hormone genes and FW entrance

A line of evidence suggested that synthesis and release of VT and PRL in pre-spawning salmon was regulated by GnRH neurons. We recently demonstrated that sGnRH directly elevated neural activities of VT neurons in the PM of pre-spawning chum salmon in the Ishikari Bay (Abe and Urano, 2005). In masu salmon, administration of GnRH analogue

elevated the amounts of PRL mRNA in spring, when wild fish migrate from ocean through the natal river (Bhandari et al., 2003). These results support the notion that sGnRH neurons coordinately facilitate gene expression for osmoregulatory hormones, and in turn facilitate migratory behavior from SW through FW environment.

## 4 Conclusion

In the present article, we focused on regulation of gene expression for VT, GH and PRL, to understand molecular events that underlie osmoregulation of homing salmon (Table 1). We then introduced the data on migratory behavior from SW thorough FW. The previous results presented in the present review suggested that hypo-osmotic stimulation by transition from SW through FW was not critical to modulate expression of osmoregulatory hormone genes in pre-spawning salmon. In the SW to FW transfer experiments, expression of VT and PRL genes in pre-spawning chum salmon changed within a few days in both SW and FW environments, with loss of salinity tolerance and progress in final maturation. The temporal changes in gene expression for these osmoregulatory hormones can be predictive or preparatory for the forthcoming FW migration.

The profiles of migratory behavior from SW through FW suggested that homing salmon continued vertical movement between SW and FW environment in the mouth of the river for a while, followed by upstream migration in FW. As mentioned above, salinity tolerance is gradually attenuated with progress of gonadal maturation. Such vertical movements between SW and FW may help decrease the plasma $Na^+$ levels and maintain internal milieu favorable for the forthcoming FW migration and spawning.

Preference to FW in pre-spawning chum salmon was related to circulating levels of sex steroid hormones. This finding supports our hypothesis that sGnRH neurons concordantly facilitate upstream migratory behavior and final gonadal maturation (Onuma et al., 2005b).

A line of previous evidence suggests that GnRH and sex steroid hormones modulate synthesis and release of VT and PRL in pre-spawning salmon. The neuroendocrine systems that govern salt and water homeostasis and migratory behavior from SW through FW should profoundly interact with the HPG-axis in pre-spawning salmon.

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Table 1. A summary of changes in expression of genes encoding osmoregulatory hormones during homing migration in pre-spawning chum salmon.

| | Proposed function | Lower ambient temp | | Higher ambient temp | |
|---|---|---|---|---|---|
| | | Males | Females | Males | Females |
| Plasam $Na^+$ | | predictive decrease in SW | | decrease in FW | |
| Vasotocin gene | diuretic | unclear | marked decrease | increase | decrease |
| GH gene | SW adaptation | not consistent | | not consistent | |
| PRL gene | FW adaptation | increase following FW entrance | | predictive increase in SW | |
| SL gene | reproduction | gradual increase | | gradual increase | |

GH, growth hormone; PRL, prolactin; SL, somatolactin; FW, fresh water; SW, seawater

**Figure Legends**

Figure 1. A profile of upstream migratory behavior shown by ambient salinity and swimming depth of the fish, which was recaptured at location F in figure 4, 14 days after the release. (A) A full record of migratory behavior until the fish was recaptured. Considering the change in water salinity, the fish entered FW environment about two days after the release (arrow). (B) A profile of migratory behavior for three days after the release (data in the boxed time period in (A) is expanded). Note that the fish spent most of time in the surface blackish layer in SW environment before entering the river. The fish continued vertical movement between SW and FW environment at the entrance of FW environment for several hours, followed by further upstream migration into FW.

Figure 2. Examples of changes in the plasma levels of T in aquarium experiment in 2002. Similar experiments were carried out in both 2001 and 2002. Data points are expressed as a pie chart which indicates the ratio of time when the fish spend in FW (white) and in SW (color) for 24 hours before collection of blood sample. Note that, in both sexes, individuals with lower plasma T levels stayed longer in FW than in SW compared to the fish mainly stayed in SW.

Figure 3. Changes in the plasma levels of T and E2 during upstream migration from coast through the natal hatchery from 2001 to 2005. Each value represents mean ± standard error ($n \geq 7$). To avoid complexity, significant differences among sampling points identified with different letters are shown only in 2005 ($p < 0.05$ by one-way ANOVA following Turkey's test), when migratory behavior was investigated by use of data loggers as is shown in Fig. 1. Sampling areas below the X-axis are the same as previously described.

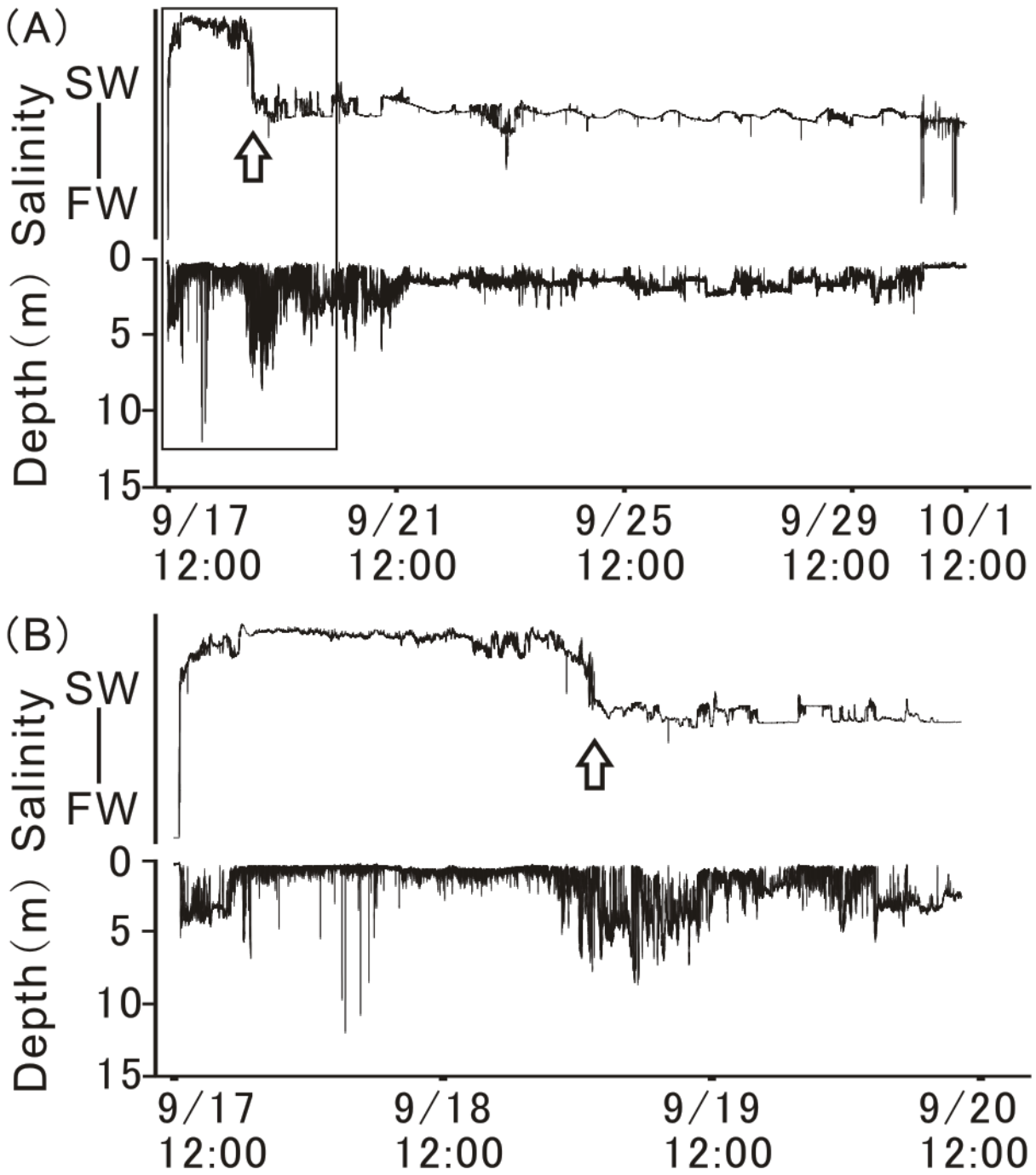
(A)
Salinity
SW
FW
Depth (m)
0
5
10
15
9/17 12:00
9/21 12:00
9/25 12:00
9/29 12:00
10/1 12:00
(B)
Salinity
SW
FW
Depth (m)
0
5
10
15
9/17 12:00
9/18 12:00
9/19 12:00
9/20 12:00

Figure 1

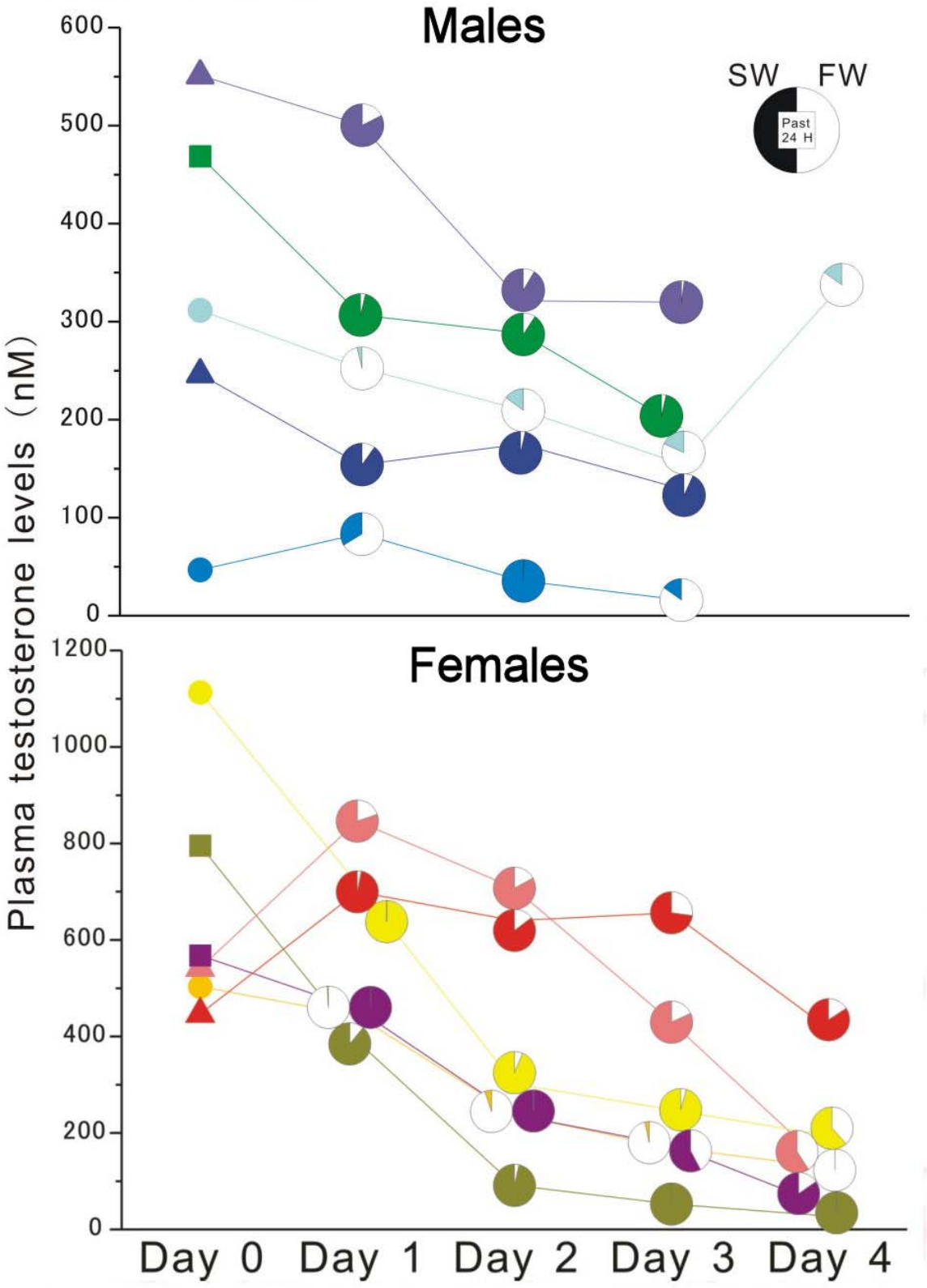
Males
SW
FW
Past
24 H
600
500
400
300
200
100
0
Females
1200
1000
800
600
400
200
0
Plasma testosterone levels (nM)
Day 0
Day 1
Day 2
Day 3
Day 4

Figure 2

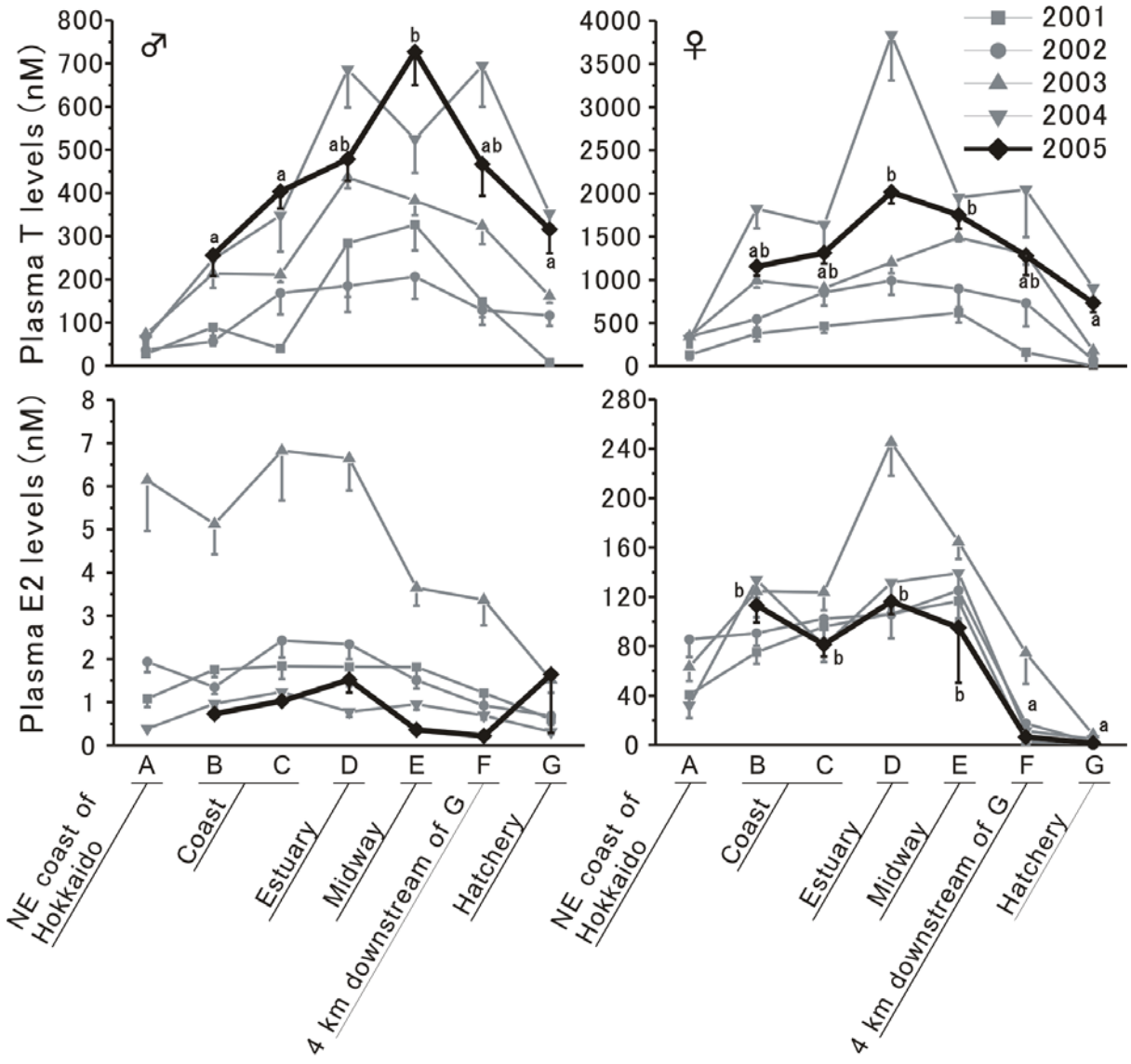
♂
♀
2001
2002
2003
2004
2005
Plasma T levels (nM)
Plasma E2 levels (nM)
A
B
C
D
E
F
G
NE coast of Hokkaido
Coast
Estuary
Midway
4 km downstream of G
Hatchery

Figure 3