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Immunocytochemical Analysis of Pituitary Cells in Pre-spawning Chum Salmon

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ABSTRACT—Pituitary hormones regulate various physiological functions during spawning migration in salmonid. Cytological features of pituitary cells were therefore immunocytochemically examined by use of antisera against homologous hormones in pre-spawning chum salmon (*Oncorhynchus keta*) caught from Ishikari Bay and the Chitose River in October, 1996. Immunoreactivity and sizes of pituitary cells were determined by a computer-aided image analyzing technique. Immunoreactivity in growth hormone (GH) cells was stronger with enlarged cell sizes in freshwater (FW) fish than in seawater (SW) ones of both sexes. Majority of prolactin (PRL) cells also had significantly stronger immunoreactivity with enlarged cell sizes in FW fish than in SW animals of both sexes. Immunoreactivity in somatolactin (SL) cells was markedly stronger with enlarged cell sizes in FW fish than in SW ones of both males and females. In addition, greater portions of SL cells were strongly stained in FW animals than in SW ones. A greater portion of gonadotropin (GTH) I cells had stronger immunoreactivity with reduced cell sizes in FW fish of both sexes, when compared with SW fish. Conversely, GTH II cells had significantly stronger immunoreactivity with enlarged cell sizes in FW ones of both sexes. In proopiomelanocortin (POMC)-derived hormone producing cells, adrenocorticotropin (ACTH) cells had stronger immunoreactivity in FW animals of both sexes, while cell sizes did not change. In melanotropes, the cells immunoreactive to α-melanophore stimulating hormone (α-MSH) antiserum had stronger immunoreactivity with reduced cell sizes only in FW males, while the cells immunoreactive to β-endorphin antiserum had stronger immunoreactivity with reduced cell sizes in FW fish than in SW animals of both sexes. Implication of these results was discussed along with previous reports on gene expression of pituitary hormone precursors.

INTRODUCTION

The pituitary gland of salmonids serves major endocrine function by secreting a variety of hormones that play vital roles in all aspect of life cycle including somatic growth, sexual maturation, reproduction, spawning migration and environmental adaptation. In teleosts, as in other vertebrates, different types of pituitary cells have been identified using histochemical and immunocytochemical techniques in many species, such as sockeye salmon, *Oncorhynchus nerka* (McKeown and van Overbeeke, 1971; Nagahama, 1973); chum salmon, *O. keta* (Nagahama, 1973; Naito *et al*., 1993a); goldfish, *Carassius auratus*, medaka, *Oryzias latipes*, eel, *Anguilla japonica* (Nagahama, 1973); some teleost species (Ball and Baker, 1969); molly, *Poecilia latipinna* (Batten and Ball, 1975; Batten, 1986); and seabass, *Dicentrarchus labrax* (Cambre *et al*., 1986). These studies reported identification and localization of different types of pituitary cells, and established that each type of pituitary cells synthesizes particular hormone for particular functions.

Among the pituitary hormones, growth hormone (GH), prolactin (PRL) and somatolactin (SL) belong to the same hormone family due to their structural similarity (Ono *et al*., 1990; Takayama *et al*., 1991; Rand-Weaver *et al*., 1991). GH and PRL are present throughout the vertebrates (Kawauchi and Yasuda, 1989a), while SL is unique in teleosts (Rand-Weaver and Kawauchi, 1993; Dores *et al*., 1996). In teleosts, GH is involved in somatic growth (Donaldson *et al*., 1979), PRL in freshwater adaptation (Hirano, 1986) and SL in osmoregulation as well as final maturation (Rand-Weaver *et al*.,

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1992; Rand-Weaver and Swanson, 1993; Olivereau and Rand-Weaver, 1994a; Kakizawa *et al.*, 1995). The levels of GH/PRL/SL family mRNAs were elevated in freshwater (FW) chum salmon compared with seawater (SW) ones during spawning migration (Taniyama *et al.*, 1999). Further, the plasma levels of GH/PRL/SL family hormones were higher in FW fish than in SW ones during homing migration in chum salmon (Kakizawa *et al.*, 1995). Therefore, cytological analyses of GH/PRL/SL family cells are important for considering changes in cellular activity during spawning migration.

Teleosts have two gonadotropins (GTH), referred to as GTH I and GTH II (Suzuki et al., 1988; Kawauchi *et al.*, 1989b; Swanson *et al.*, 1991). They are produced separately in distinct cells which differentially expressed the gene for β-subunit of each GTH (Naito *et al.*, 1991). GTH I is responsible for early stages of gonadal development like tetrapod follicle-stimulating hormone (FSH), while GTH II stimulates final maturation like tetrapod luteinizing hormone (LH). The levels of GTH Iβ mRNA did not change during homing migration in chum salmon, while GTH IIβ mRNA considerably increased in FW fish of both sexes (Kitahashi *et al.*, 1998). The plasma levels of GTH II also increased at the final stage of maturation in teleost fishes, as reported in striped bass (Mylonas *et al.*, 1997) and rainbow trout (Sumpter and Scott, 1989; Gomez *et al.*, 1999). However, there are few reports on cytological features of GTH I and II cells during spawning migration.

Adrenocorticotropic hormone (ACTH), α-melanophore-stimulating hormone (α-MSH) and β-endorphin are derived from a common protein precursor proopiomelanocortin (POMC) through proteolytic cleavages in all Gnathostomata. It is considered that ACTH cells are activated during stressful stimuli (Anguileria, 1994; Sumpter *et al.*, 1986; Balm and Pottinger, 1995), while α-MSH cells for skin coloration (Lamacz *et al.*, 1991). Cytological studies of POMC-derived hormone producing cells have done in many teleost species such as rainbow trout (Rodrigues and Sumpter, 1984), Mediterranean yellowtail (Garcia-Hernandez *et al.*, 1997) and primitive actinopterygians (Joss *et al.*, 1990). However, studies on cytological changes of POMC-derived hormone producing cells during spawning migration are very limited. The present study therefore included analyses of ACTH cells and α-MSH cells which also contain β-endorphin immunoreactivity to see whether they have significant roles during spawning migration in salmonids.

In the present study, changes in cytological features of pituitary cells were immunocytochemically examined for better understanding of endocrine events in homing salmon. Pre-spawning chum salmon were obtained from the coastal sea and at the hatchery. Tissue sections were stained with antisera against homologous hormones. Hence, we did not immunostain thyroid-stimulating hormone (TSH) cells, because anti-salmon TSH was unavailable.

MATERIALS AND METHODS

Fish

Pre-spawning chum salmon (*Oncorhynchus keta*) of both sexes in the final stages of spawning migration were caught at Atsuta, a fishermen's village facing the Ishikari Bay, and Chitose, a town through which a branch of the Ishikari River runs, in October, 1996. The fish caught at Chitose were maintained at the Chitose Salmon Hatchery (Hokkaido, Japan) until fully matured. The sampling sites are the main key points along the migratory route of Ishikari stock chum salmon. Since the Ishikari is a prominent big river, and a vast number of juveniles are released only from the Chitose Salmon Hatchery, the salmon caught at Atsuta and Chitose can be considered to belong to the same genetic population. The animals caught at Atsuta are referred to as seawater (SW), and those captured at Chitose as fresh-water (FW) salmon. Immediately after anesthetization with 0.02% tricaine-methane sulfonate (MS 222, Sigma), the animals were weighed and measured of body length, and then they were decapitated. The maturity of the gonads was later assessed by the gonadosomatic index (GSI, gonad weight x 100/body weight). The changes in GSI values in the SW and FW fish, an increase in females and a decrease in males, indicate that the final maturation of the gonads in the experimental fish might occur during migration from Atsuta to Chitose (Table 1).

Table 1. Body weight (kg), gonadosomatic index, GSI (%) and hepatosomatic index, HSI (%) of seawater (SW) and freshwater (FW) chum salmon of both sexes used in the present study.

Sex	Sampling site	N	Body weight (kg)	GSI (%)	HSI (%)
♂	Atsuta (SW)	3	3.9±0.3	5.4±0.3	1.3±0.2
	Chitose (FW)	3	3.2±0.8	4.2±0.4	1.9±0.1
♀	Atsuta (SW)	3	2.7±0.3	12.0±1.5	2.6±0.5
	Chitose (FW)	3	3.1±0.1	23.2±1.4	1.2±0.1

Tissue preparation

The pituitary glands were collected by the decapitation after anesthetization with 0.02% MS-222 and were fixed in phosphate buffered 4% paraformaldehyde (pH 7.5) at 4°C overnight. The samples were dehydrated through a series of graded ethanols and were embedded in paraplast. Tissue blocks were transversely sectioned at 8 μm thickness. The sections were then divided into eight parallel sets in a manner that peripheral, sub-medial and medial sections should be mounted on single gelatinized slides.

Immunocytochemistry

Pituitary cells were immunostained with a Vectastain ABC (Avidin-Biotin-Peroxidase Complex) kit following the manufacturer's instructions with slight modification. In brief, the sections were deparaffinized, rehydrated through graded ethanols, and were washed in a phosphate-buffered saline (PBS, 0.01 M, pH 7.5). The rehydrated sections were pre-incubated in 1% normal goat serum in PBS in a moist chamber at room temperature (RT) for 30 min. The tissue sections were then incubated with a primary antiserum in the moist chamber at 4°C for 48 hr. Afterward, they were washed in PBS three times, five minutes each, and were incubated in a solution of biotinylated anti-rabbit IgG at RT for 30 min followed by ABC complex (30 min at RT). After wash in tris-buffer (TB, pH 7.5, 0.01 M), the sections were immersed in a DAB solution (3, 3' diaminobenzidine tetrahydrochloride, 0.1%; H2O2 , 0.02%; tris-buffer, 0.05 M, pH 7.5) at 20°C for 10 min, rinsed in deionized water, and were coverslipped with permount.

Specificity of antisera (GH, GTH Iβ and GTH IIβ) were confirmed by an absorption test using chum salmon GH, GTH Iβ and GTH IIβ provided by Prof. H. Kawauchi, Kitasato University, Japan. Immunostaining was completely prevented by absorption with the corre-

sponding antigen of chum salmon GH, GTH Iβ and GTH IIβ (74.25 ng/ml). Absorption tests were not carried out for anti-PRL, anti-SL, anti-ACTH, anti-α-MSH and anti-β-endorphin, since their specificity were well confirmed by the researchers who provided these antisera.

The pituitary sections from SW and FW animals were immunostained in the same run to ensure the same experimental condition between groups, since immunoreactive differences were accounted for analysis.

The optimal dilutions of primary antisera were determined by a preliminary experiment using a series of serially diluted antisera. The antisera used in the present study were anti-chum salmon GH and PRL, 1:64000; anti-chum salmon SL, 1:10000; anti-chum salmon GTH Iβ and IIβ, 1:16000; anti-kokanee salmon ACTH, 1:8000; anti α-MSH and anti-β-endorphin, 1:4000. The antisera were diluted with PBS containing 0.5% bovine serum albumin (BSA). Backgrounds of immunostained sections were carefully checked since false staining may occur due to endogenous biotin in animal tissues (Wang and Pevsner, 1999).

The rabbit anti-chum salmon GH antiserum (Lot No. 8502), GTH Iβ (Lot No. 8510) and GTH IIβ (Lot No. 8506) were provided by Prof. H. Kawauchi, Kitasato University, Japan. Anti-chum salmon PRL and SL were prepared by Prof. T. Kaneko, Tokyo University, Japan. Anti-kokanee salmon ACTH, α-MSH and β-endorphin were generously provided by Prof. R. M. Dores, University of Denver, USA.

Selection of immunostained sections

Camera-lucida drawings were made using all immunostained sections to measure the sizes of areas immunoreactive to each of applied antisera. For each hormone, the section that contained the largest immunoreactive (ir) area was selected as the representative one of individual animal, and was used for later image analysis. The photomicrographs were taken from the selected section with the reversal film (Fuji film, Sensia II) by use of a x 100 objective lens of multipurpose microscope (Zeiss Axiophot, Germany). Six to eight photomicrographs of ca. 100 μm x 150 μm areas were taken from the anterior, posterior, dorsal, ventral and medial parts of the selected sections to avoid deviation of analyzed cells from the whole population of each type of pituitary cells.

Image analysis

The photomicrographs were scanned with a film scanner (Polaroid Scanner, Polaroid Corp., USA) at 1350 dpi for image analysis. All recognizable immunoreactive cells were analyzed from the selected photomicrograph as mentioned above. Immunoreactivity and sizes of all recognizable immunoreactive cells on the scanned film were measured by a computer-aided Image Pro-Plus software (Media Cybernetics, USA). Then, cell sizes were calibrated as μm^2 from the number of pixels. Magnitudes of immunoreactivity were expressed as arbitrary units of gray scale after subtraction of background from the nearby unstained cells.

Statistical analysis

Data are expressed as mean±SEM. After *F*-test for variance, Welch t-test was used to test the differences between SW and FW animals for immunoreactivity and cell sizes. The correlation between cell sizes and immunoreactivity was analyzed by linear correlation method. When immunoreactive cells shown in a frequency distribution histogram of immunoreactivity or cell sizes seemed to be composed of multiple populations, the presence of the number of peaks in the histogram was analyzed by use of a computer software for Gauss Model (Microcal Origin, Microcal Software Inc., USA).

RESULTS

Localization of pituitary cells

In the pituitary of pre-spawning chum salmon, PRL-ir cells were localized in the rostral pars distalis (RPD) (Fig. 1), organized in a follicular form both in SW and FW animals. GH, GTH I, GTH II and ACTH cells were localized in the proximal pars distalis (PPD), while SL, α-MSH and β-endorphin cells were localized in the pars intermedia (PI) (Fig. 1). The cells in the PPD (GH, GTH I and II) showed scattered distribution pattern instead of distinct glandular form. The distribution pattern of these hormonal cells coincided well with the previous reports cited in Introduction, indicating that the antisera used in the present study specifically stained the particular cell type.

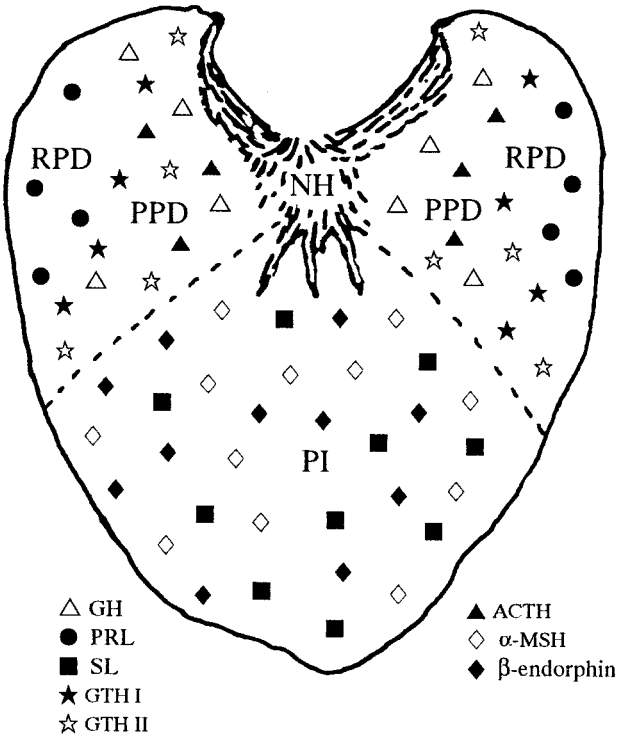

Fig. 1. Schematic diagram of a transverse section of the pituitary gland in mature chum salmon showing the localization of different types of pituitary cells. Abbreviations: RPD, rostral pars distalis; PPD, proximal pars distalis; PI, pars intermedia; NH, neurohypophysis; GH, growth hormone; PRL, prolactin; SL, somatolactin; GTH, gonadotropin; ACTH, adrenocorticotropic hormone; α-MSH, α-melanophore-stimulating hormone.

GH cells

Frequency distribution histograms of immunoreactivity in GH cells in the males and FW females (Figs 2A, B and D) indicate that GH cells in these pre-spawning chum salmon are composed of a single cell population. However, the histogram of GH cells in the SW females (Fig 2C) shows the presence of two cell populations, weakly stained and strongly stained (tested by Gauss Model, figure not shown), as was seen in the juvenile chum salmon that were reared in a low population density and showed a better growth rate than those in a high population density (Salam *et al.*, 1999). Immunoreactivity in GH cells was stronger ($p<0.001$) in FW males than in SW males (Figs 2A and B), while the structure of GH cell populations changed in females during the last phases of

spawning migration. The weakly stained cell population seen in SW females was markedly decreased in FW females (Figs 2C-D). Such change resulted in a slight but significant increase ($p<0.01$) in GH immunoreactivity in FW females compared with SW females (Figs. 2C-D). The sizes of GH-immunoreactive (ir) cells were slightly enlarged in FW males (Figs. 2E and F, $p<0.01$) and females (Figs. 2G and H, $p<0.05$) than in SW ones.

PRL cells

PRL-ir cells in FW fish were more strongly immunostained than those in SW fish (Figs. 3A and B). The skewed patterns

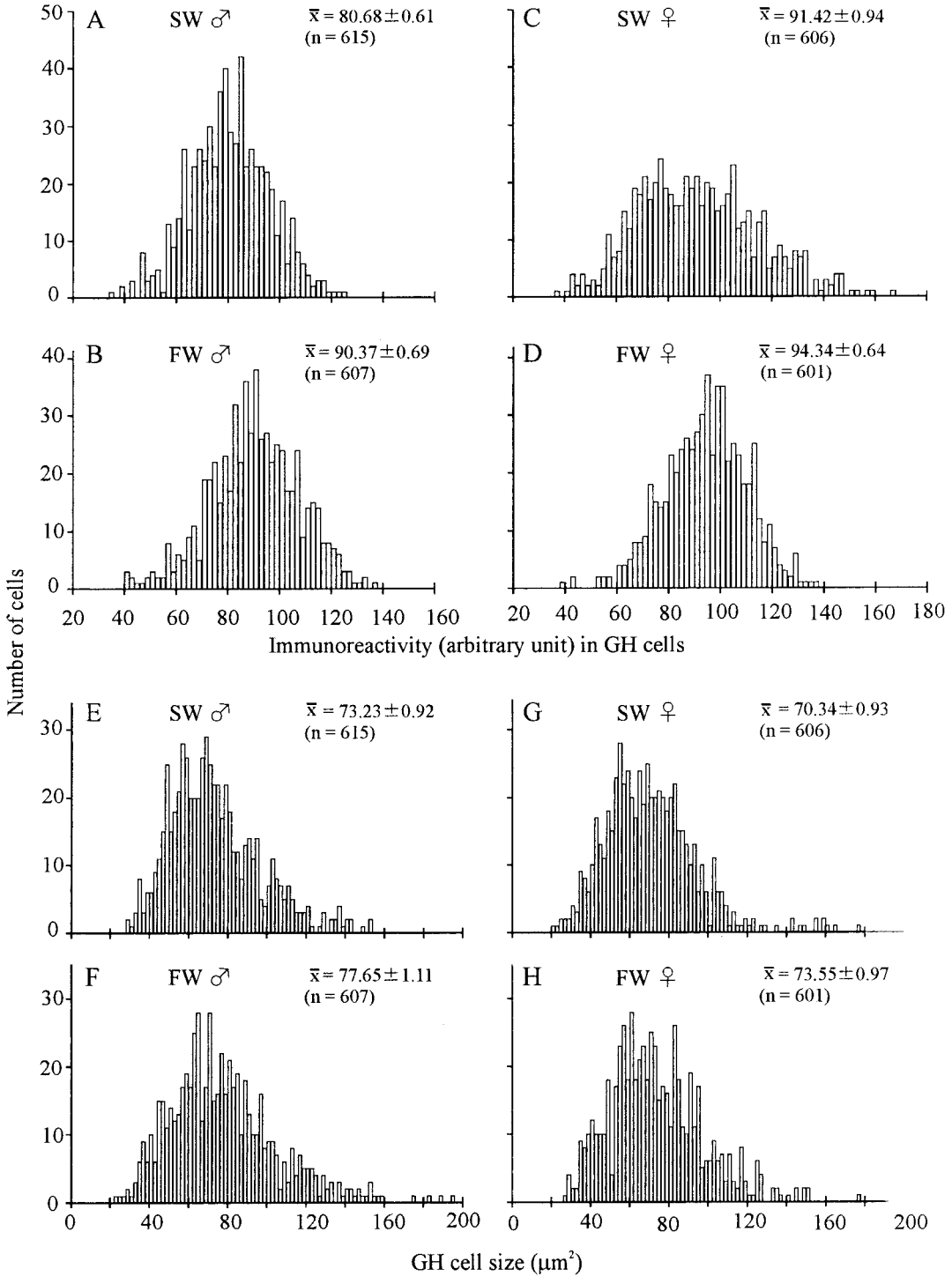

Fig. 2. Frequency distribution histograms of immunoreactivity (A-D) and cell sizes (E-H) of GH-ir cells in the pituitaries of SW and FW fish of both males and females.

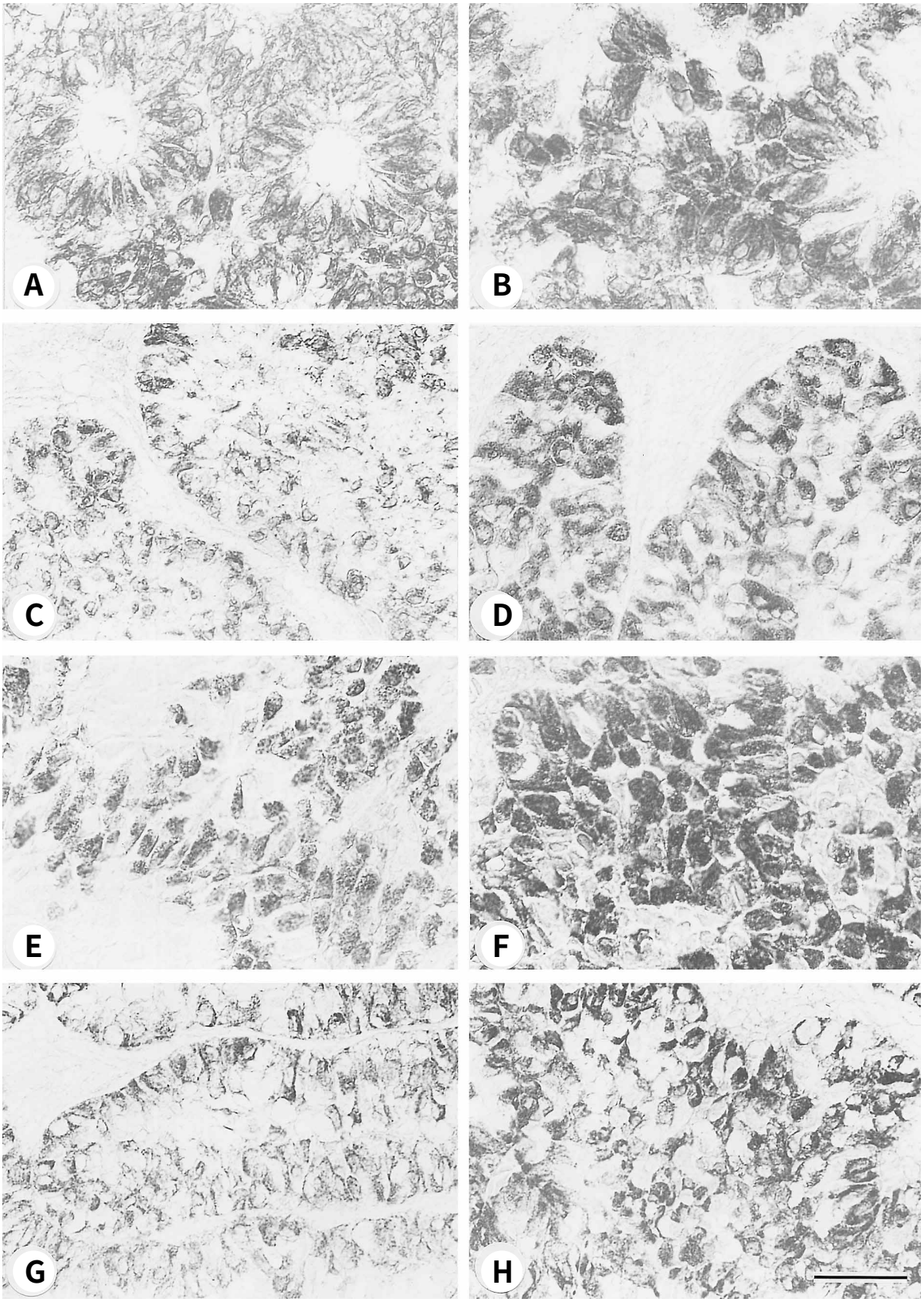

Fig. 3. Photomicrographs of PRL (A and B), SL (C and D), GTH II (E and F) and β-endorphin (G and H) cells in the pituitaries of SW (A, C, E and G) and FW (B, D, F and H) female chum salmon. Note the strongly stained cells in FW animals (right panel). Differences in immunoreactivity of these hormonal cells between SW and FW males were similar as shown in these photomicrographs (figures not shown). Scale bar, 40 μm.

of frequency distribution histograms of PRL immunoreactivity and cell sizes may show that PRL cells are composed of dominant and multiple small populations, although the peaks were single (Figs 4A-H). These PRL cell populations similarly behaved during the last phases of spawning migration. Actually, the distribution histograms of immunoreactivity in FW fish of both sexes concordantly shifted towards the right compared with those in SW fish, resulted in the fact that almost all PRL cells were strongly stained in FW fish (Figs. 4A vs B, and C vs D). In both males and females, PRL-ir cells thus had significantly ($p<0.001$) stronger immunoreactivity in FW fish than in SW ones. In addition, PRL-ir cell sizes enlarged significantly ($p<0.001$) in FW animals than in SW fish of both sexes (Figs. 4E vs F, and G vs H). The distribution histograms of PRL cell sizes shifted towards the right in a coordinated manner, and showed that the pituitaries of FW fish contained greater num-

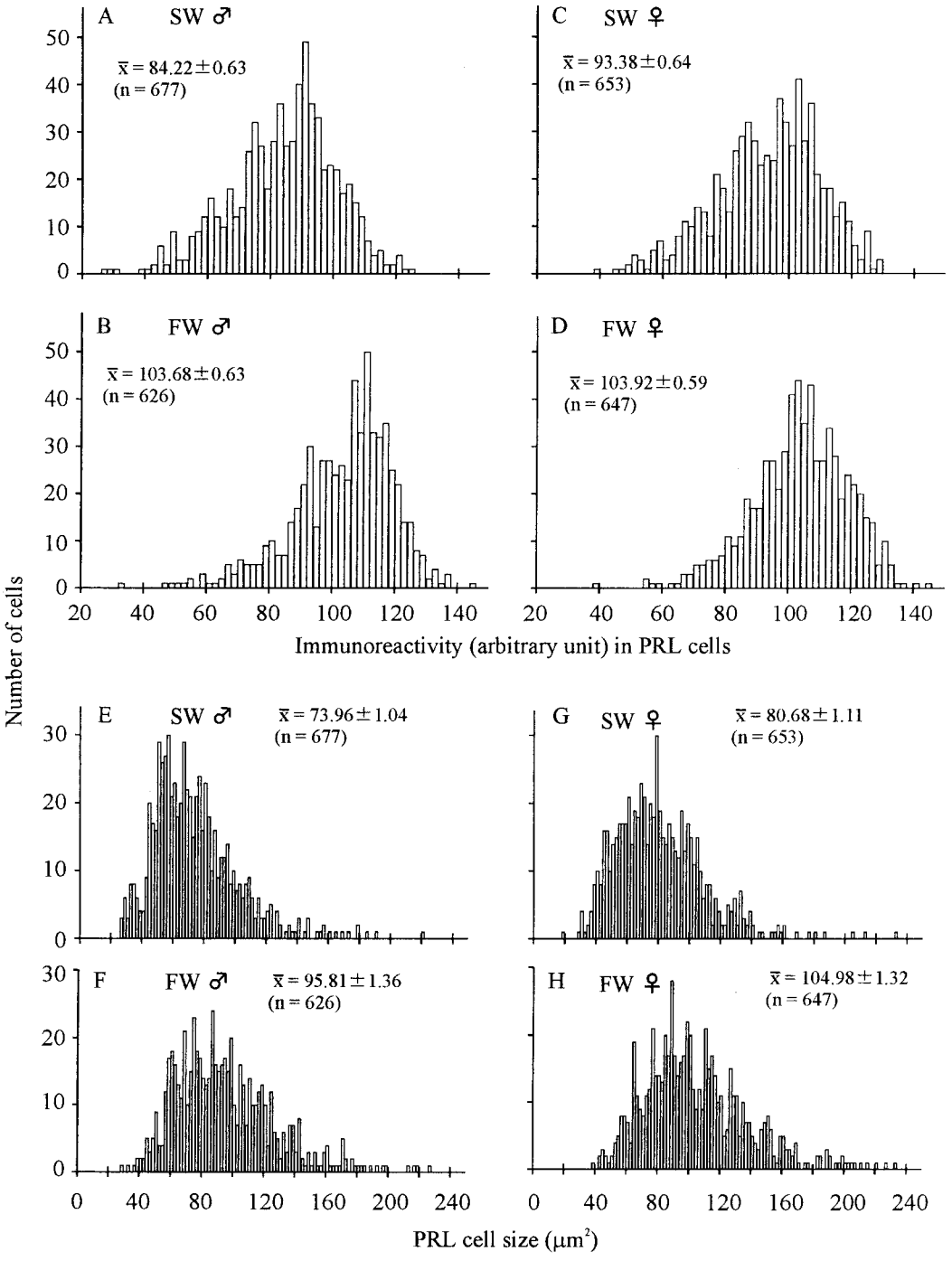

Fig. 4. Frequency distribution histograms of immunoreactivity (A-D) and cell sizes (E-H) of PRL-ir cells in the pituitaries of SW and FW fish of both males and females. Note that greater numbers of PRL cells in FW fish of both sexes are strongly stained.

bers of larger PRL cells compared with the SW ones (Figs. 4E-H). An analysis by scatter plot distributions further revealed that the numbers of strongly stained larger cells were greater in FW animals regardless of sexes (figure not shown).

SL cells

Although the frequency distribution histograms of SL immunoreactivity and cell sizes were skewed, major populations of SL cells similarly increased their immunoreactivity and cell sizes during the upstream migration. Immunoreactivity in SL-ir cells in FW fish was thus stronger than that in SW fish of both sexes ($p<0.001$) (Figs. 3C and D, 5A-D). Frequency distribution histograms of immunoreactivity indicated that almost all SL cells had stronger immunoreactivity in FW fish (Figs.

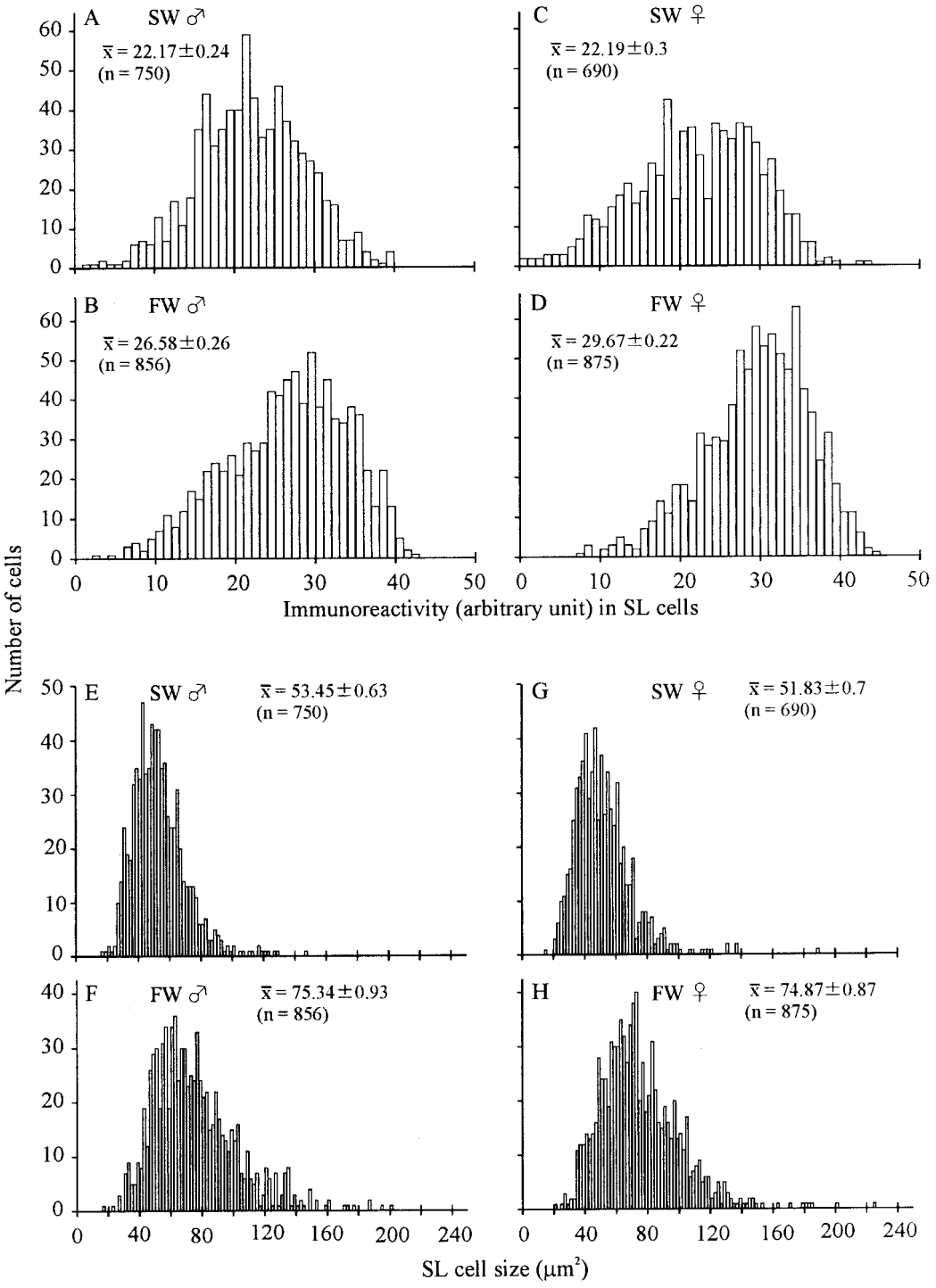

Fig. 5. Frequency distribution histograms of immunoreactivity (A-D) and cell sizes (E-H) of SL-ir cells in the pituitaries of SW and FW fish of both sexes. Note that greater numbers of SL cells in FW fish of both sexes are strongly stained.

5B and D). SL-ir cell sizes also enlarged in FW animals compared with SW ones of both sexes ($p<0.001$) (Figs.5E-H). Histograms of cell sizes were broaden towards the right, indicating that the numbers of larger cells were greater in FW animals of both sexes (Figs. 5F and H). Since scatter plot distribution showed that the numbers of strongly stained larger SL cells were greater in FW animals when compared with those in SW fish of both sexes (arrows in Figs. 6B and D), it is apparent that a considerable number of SL cells concomitantly increased their immunoreactivity and sizes.

GTH I cells

GTH Iβ-ir cells had significantly stronger immunoreactivity in FW fish than in SW animals of both sexes ($p<0.001$) (Figs. 7A-D). Distribution histograms of immunoreactivity indicate the presence of greater numbers of strongly stained GTH I cells in FW fish of both sexes (Figs. 7B and D). The rightward shift of the histogram in FW females was more prominent than that in FW males. Whereas, GTH I cell sizes significantly reduced in FW fish than in SW fish of both sexes ($p<0.001$) (Figs. 7E-H). The changes in the shapes of histograms of GTH-I cell sizes were the most drastic among all pituitary cells examined in the present study. A scatter plot distribution analysis between immunoreactivity and cell sizes showed that smaller cells predominant in FW animals were strongly immunostained (figure not shown).

GTH II cells

In GTH IIβ-ir cells, immunoreactivity was stronger in FW fish than in SW ones in both sexes ($p<0.001$)(Figs. 3E and F, 8A-D). Distribution histograms of immunoreactivity showed that a broad distributional pattern with multiple peaks in SW fish was transformed into a single peak Gaussian pattern, which was composed of a greater numbers of strongly stained GTH II cells, in FW fish of both sexes (Figs. 8B and D). GTH IIβ-ir cell sizes also were significantly larger ($p<0.001$) in FW fish

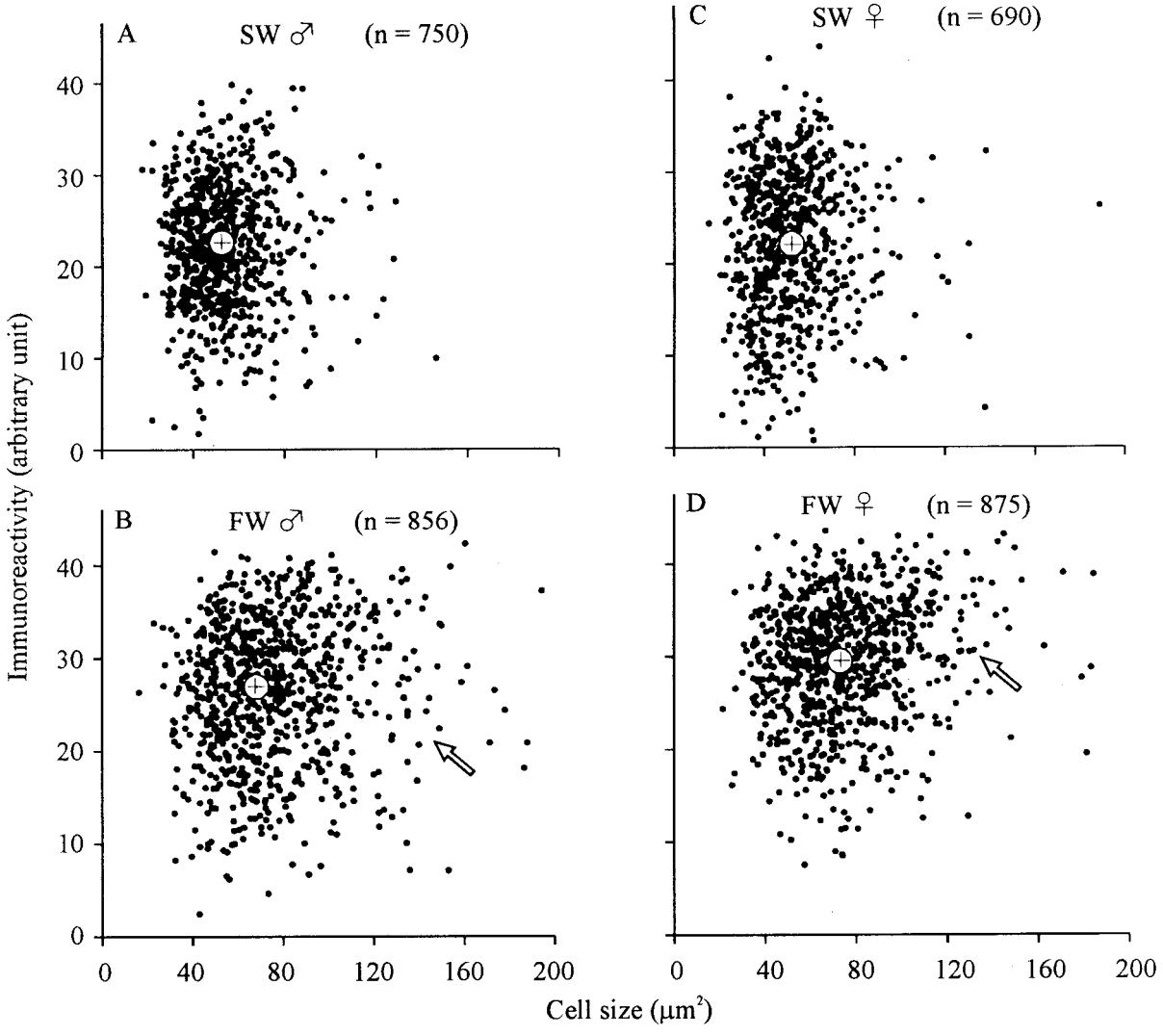

Fig. 6. Scatter plot distributions between immunoreactivity and cell sizes of SL-ir cells in the SW and FW males (A and B) and females (C and D). Note that larger SL cells in FW fish show stronger immunoreactivity. Standard deviations are shown in the white circle. Arrows indicate the presence of cell populations of strongly stained larger cells.

than in SW ones of both sexes (Figs. 8E-H). Histograms of cell sizes shifted towards higher values in FW fish, indicating the presence of greater numbers of larger cells in FW animals compared with SW ones (Figs. 8F and H). Scatter plot analysis showed that, in contrast to GTH Iβ-ir cells in FW fish which were small and strongly immunoreactive, larger GTH-IIβ ir cells in FW fish were strongly stained, when compared with GTH IIβ-ir cells in SW fish (figure not shown).

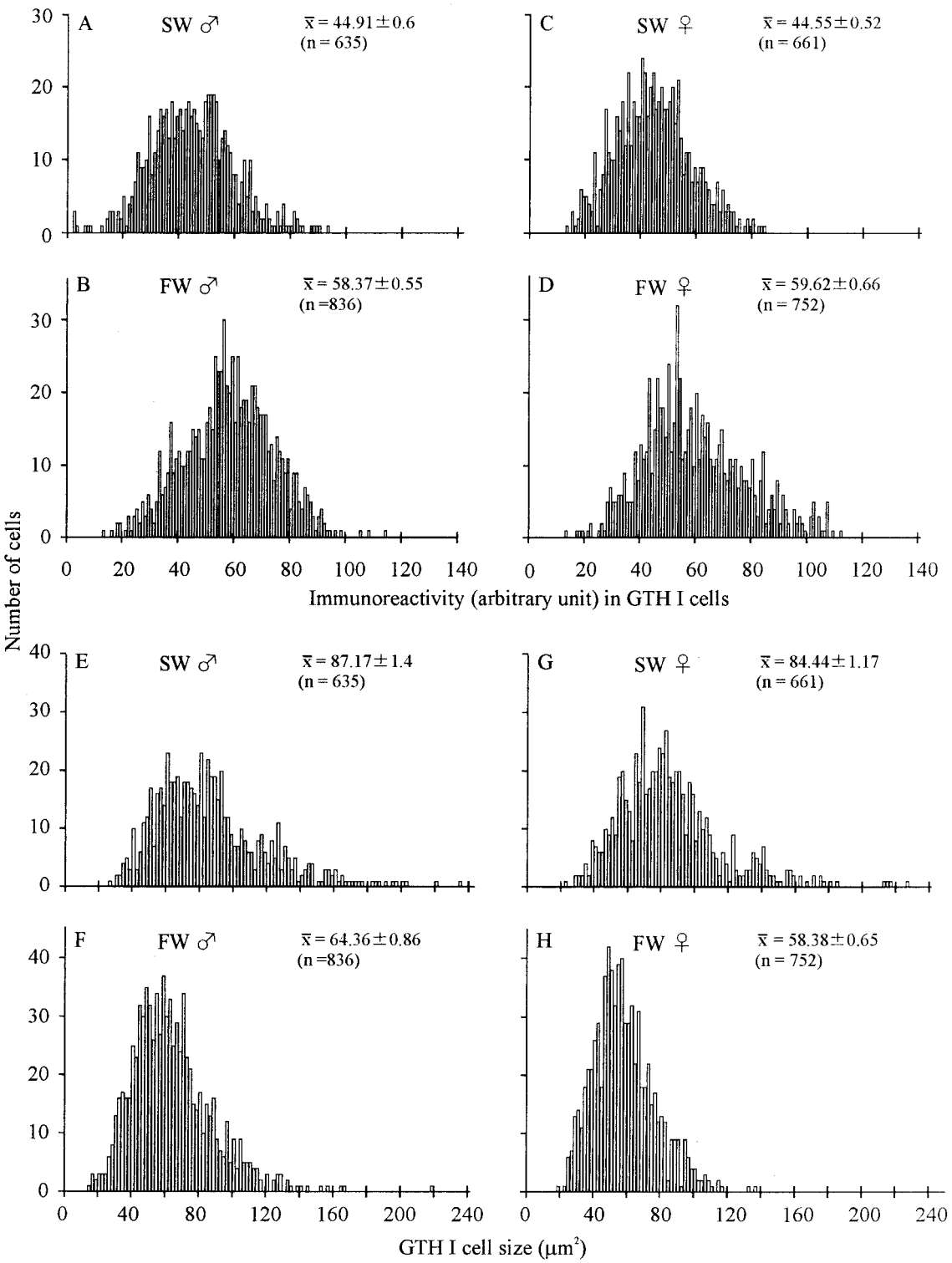

Fig. 7. Frequency distribution histograms of immunoreactivity (A-D) and cell sizes (E-H) of GTH I-ir cells in the pituitaries of SW and FW fish of both sexes. Note that greater numbers of GTH I cells in FW fish of both sexes are strongly stained.

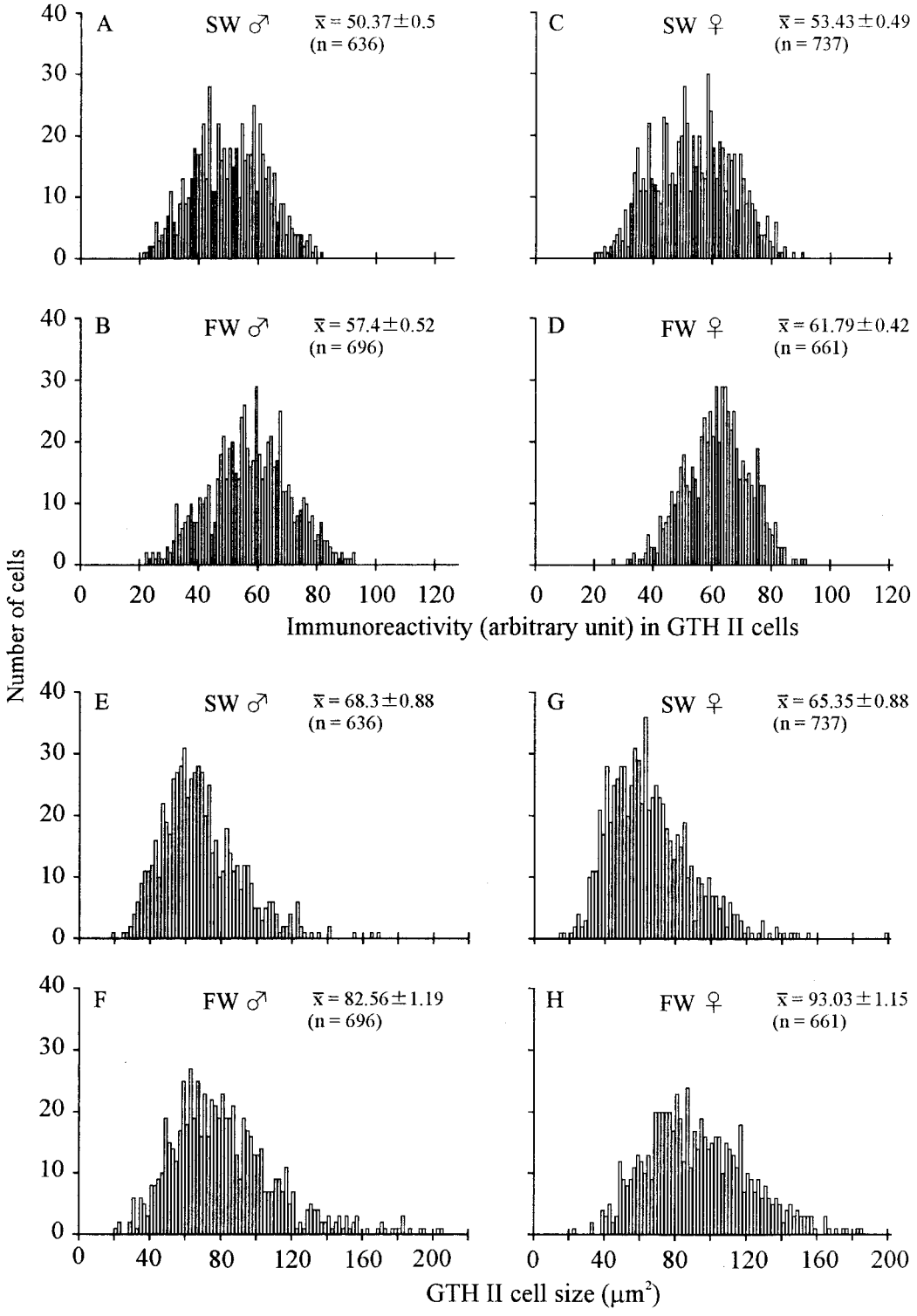

Fig. 8. Frequency distribution histograms of immunoreactivity (A-D) and cell sizes (E-H) of GTH II-ir cells in the pituitaries of SW and FW fish of both sexes. Note that greater numbers of GTH II cells in FW fish of both sexes are strongly stained.

POMC-derived hormone cells

Immunoreactivity in corticotropes (ACTH cells) in the PPD was stronger ($p<0.001$) in FW fish than in SW ones of both sexes (Figs. 9A-D), while cell sizes did not change either in males or females (Figs. 9E-H).

In the pars intermedia, the localization of α-MSH-ir cells (melanotropes) was nearly the same with that of β-endorphin-ir cells. In FW fish, intermediate cells, which were immunoreactive to α-MSH antiserum or β-endorphin antiserum, showed a similar increase in immunoreactivity ($p<0.001$), whereas their

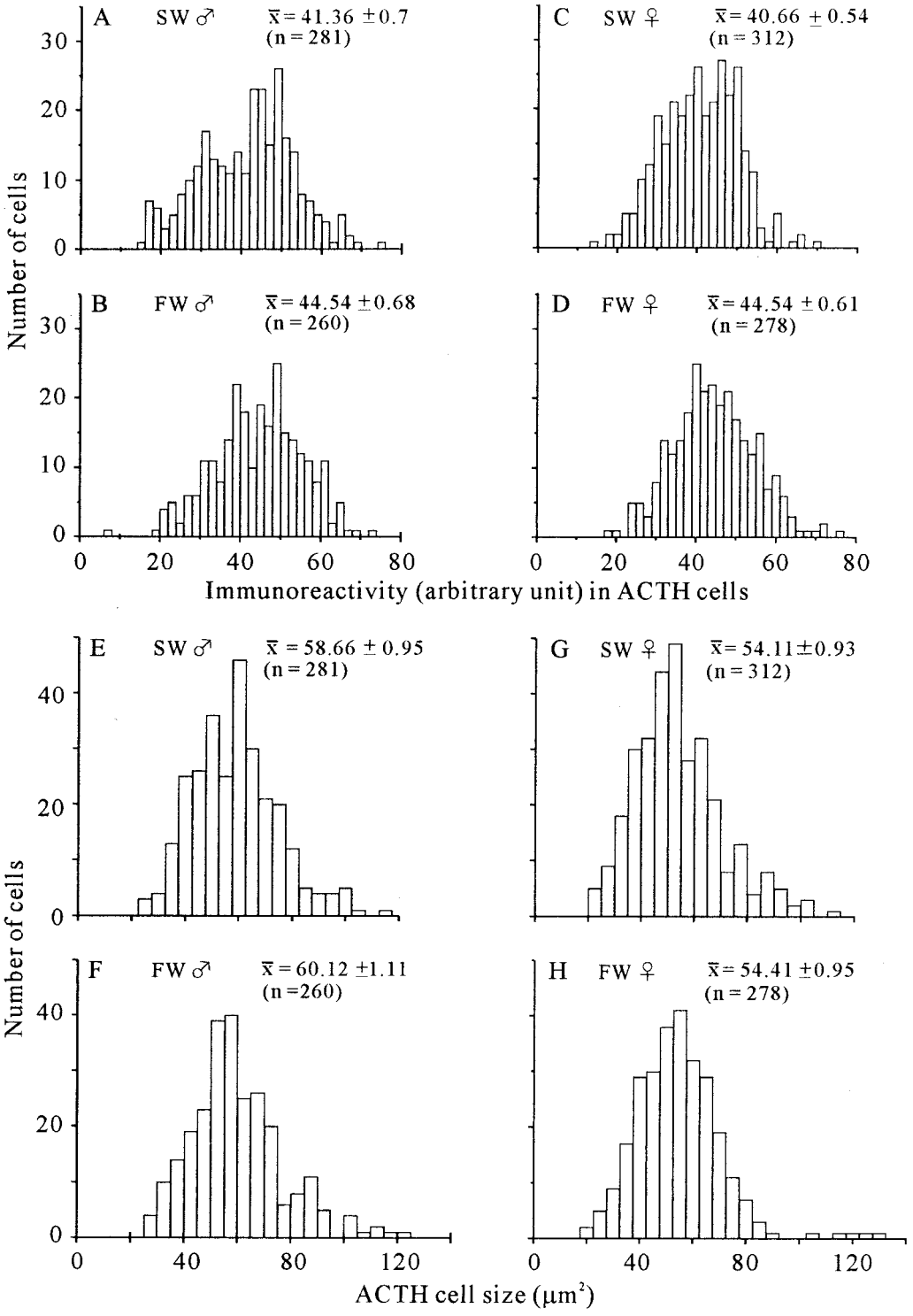

Fig. 9. Frequency distribution histograms of immunoreactivity and cell sizes of ACTH-ir cells in the pituitaries of SW and FW fish of both males and females.

cell sizes reduced ($p<0.001$), as shown in Figs. 10A-H for β-endorphin-ir cells. We considered that the present results on α-MSH-ir cells and β-endorphin-ir cells were obtained from pituitary cells of the same population, although we did not use a double immunostaining technique to confirm the presence of the two hormones in the same individual cells.

DISCUSSION

The present study analyzed GH/PRL/SL, GTHs (I and II)

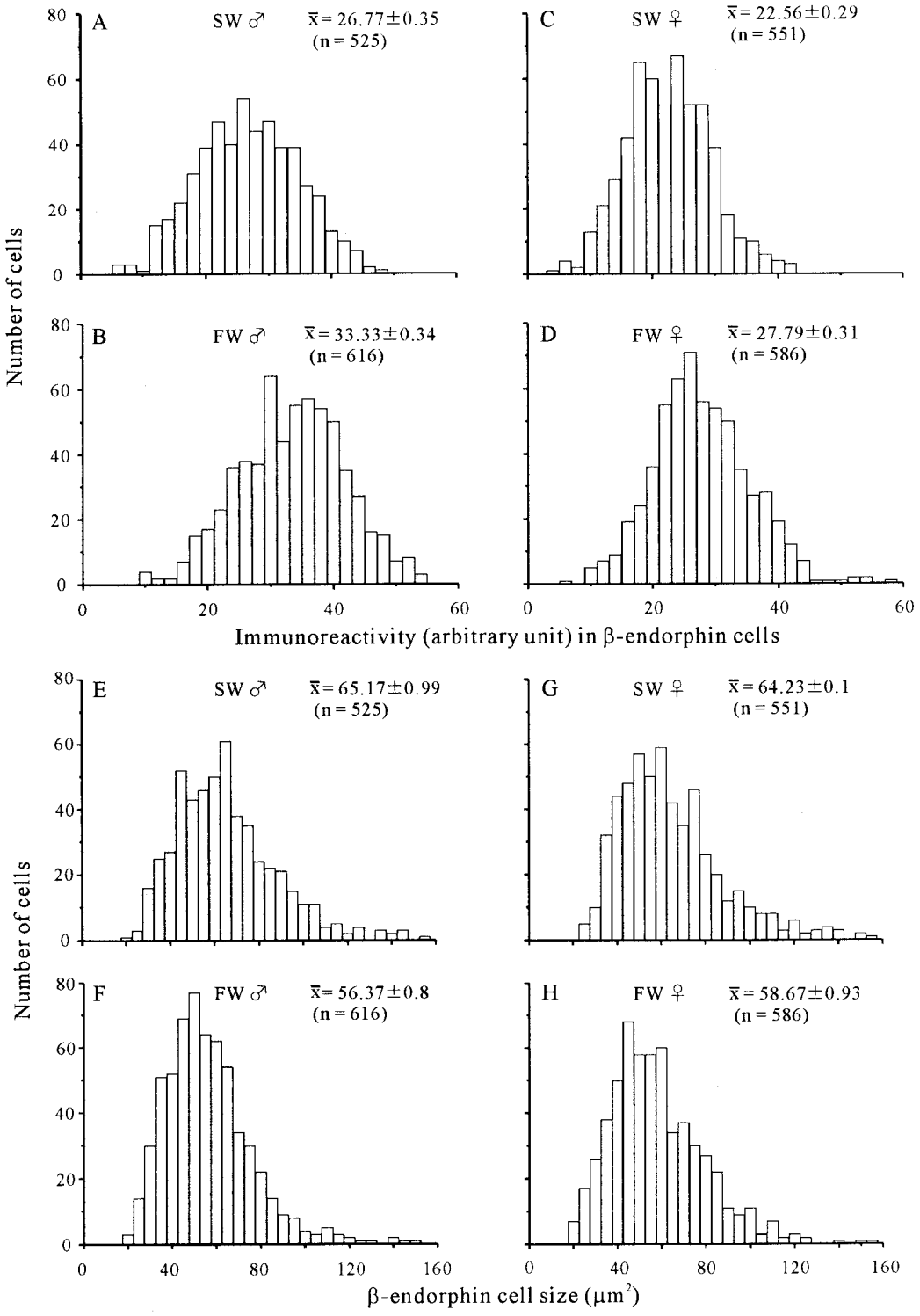

Fig. 10. Frequency distribution histograms of immunoreactivity and cell sizes of β-endorphin-ir cells in the pituitaries of SW and FW fish of both sexes. Note that greater numbers of cells are strongly stained in FW fish of both sexes.

and POMC-derived hormonal cells in the pituitaries of chum salmon during the final stages of spawning migration. We found that GH/PRL/SL family cells had stronger immunoreactivity with enlarged cell sizes in FW fish than in SW ones. In gonadotropes, we found that GTH I cells had stronger immunoreactivity with reduced cell sizes in FW animals than in SW ones, while GTH II cells had stronger immunoreactivity with enlarged cell sizes in FW animals when compared with SW ones. In POMC-derived hormonal cells, ACTH cells (corticotropes) had slightly higher immunoreactivity in FW animals than in SW

ones, but cell sizes did not change. On the other had, the melanotropes immunoreactive to α-MSH antiserum or β-endorphin antiserum had stronger immunoreactivity with reduced cell sizes in FW fish than in SW ones of both sexes.

The findings in GH/PRL/SL family cells in homing chum salmon coincide well with the previous reports that the levels of GH, PRL and SL mRNAs increased in FW chum salmon (Taniyama *et al*., 1999). Similarly, plasma levels of GH, PRL and SL elevated in FW chum salmon (Kakizawa *et al*., 1995). Since the level of mRNA indicates the transcriptional activity and plasma hormonal levels show secretory activity of pituitary cells, it is plausible that, in pre-spawning chum salmon, over-expressed hormones were stored coincidentally with the elevation of synthetic and secretory activity in pituitary cells that produce GH/PRL/SL family hormones.

Changes in morphology and activity of GH, PRL (Power, 1992) and SL (Olivereau and Rand-Weaver, 1994b) cells were associated with the different life stages. In juvenile chum salmon, larger GH cells were weakly stained compared with smaller ones (Salam *et al*., 1999). Since GH cells which extensively secreted hormone in serum-free culture decreased in immunoreactivity with the dilation of rough endoplasmic reticulum (Yada *et al*., 1991), large but weakly immunoreactive cells should have high secretory activity. Thus, the weakly stained cell population seen in the present SW females may be the remnant of such active GH cells. The slight increase in immunoreactivity of GH cells in FW fish indicates that GH cells were not so stimulated in FW environment during spawning migration as reported in striped bass, *Morone saxatilis* (Huang and Specker, 1994).

Interestingly, remarkable changes were found in immunoreactivity and cell sizes of PRL and SL cells during spawning migration. In both PRL and SL cells, immunoreactivity was considerably stronger with enlarged cell sizes in FW fish than in SW ones of both sexes. The previous reports showed higher transcriptional activity of PRL and SL cells in FW chum salmon (Taniyama *et al*., 1999) and higher secretory activity of PRL and SL cells in homing salmon (Kakizawa *et al*., 1995). Further, in the present study the cellular amounts of hormones in PRL and SL cells were increased. We therefore consider that translational activity was much higher than secretory activity during spawning migration of chum salmon. The stimulation of PRL and SL cells might be influenced in relevance with some physiological functions. In teleosts, many studies suggested that PRL is involved in FW adaptation (Hirano, 1986), while SL has function at least for osmoregulation (Kakizawa *et al*., 1996) and final maturation (Rand-Weaver *et al*., 1992; Rand-Weaver and Swanson, 1993; Olivereau and Rand-Weaver, 1994b; Taniyama *et al*., 1999).

In gonadotropes, GTH I cells had stronger immunoreactivity with reduced cell sizes in FW fish compared with the SW ones of both males and females, while GTH II cells had stronger immunoreactivity with enlarged cell sizes in FW animals. During earlier stages of sexual maturity, GTH I cells had numerous dilated cisternae of the granular endoplasmic reticulum with a small number of secretory granules, suggesting higher secretory activity of GTH I cells in vitellogenic rainbow trout (Naito *et al*., 1993b). In addition, GTH I cells showed active synthesis and release of hormone at the early stages of gonadal maturation in salmonid (Nozaki *et al*., 1990; Naito *et al*., 1991). Large GTH I cells with weak immunoreactivity in SW animals presumably underwent somehow considerable secretion, while small GTH I cells having strong immunoreactivity in FW animals were regressed without further stimulation, as fish attained near to final maturation. This may be true, because during the final stages of spawning migration, no significant changes in the levels of GTH Iβ mRNA were seen between SW and FW chum salmon of the same stock used in the present study (Kitahashi *et al*., 1998).

In contrast to GTH I cells, GTH II cells were enlarged with stronger immunoreactivity in FW animals than in SW ones of both male and female chum salmon at the time of final maturation. This finding coincides well with the reports that GTH II cells were intensely stained in mature rainbow trout prior to spawning (Nozaki *et al*., 1990). In addition, the level of GTH IIβ mRNA was higher in FW fish than those in SW ones during the final stages of spawning migration from SW to FW in chum salmon (Kitahashi *et al*., 1998). The levels of GTH IIβ mRNA also elevated in rainbow trout during final maturation (Naito *et al*., 1991; Gomez *et al*., 1999). Further, plasma levels of GTH II markedly increased during the final stages of gonadal maturation in teleost species, such as in coho salmon (Swanson, 1991b), striped bass (Mylonas *et al*., 1997) and rainbow trout (Gomez *et al*., 1999). The plasma levels of 17α,20β-dihydroxy-4-pregnen-3-one were thus dramatically increased in FW chum salmon of both sexes of the same population that were used in the present study (Ota, 1999). Since the magnitude of immunoreaction determines the amount of stored hormone, GTH II cells in FW fish may contain much higher stored hormone than those in SW ones regardless of sexes at the final stage of maturation. We consider that the translational activity of GTH II cells was much higher than secretory activity during the final maturation in salmonids.

In POMC-derived hormone-producing cells, ACTH cells had slightly stronger immunoreactivity without changing the sizes of cells in FW animals, indicating that ACTH cells remain active to secrete hormone since rapid salinity changes can act as stressors in some teleost, such as *Oreochromis mossambicus* (Balm *et al*., 1994), and *Sparus aurata* (Mancera *et al*., 1994). Such possibility in pre-spawning chum salmon is supported by the fact that plasma cortisol levels were dramatically elevated in the same population of homing chum salmon used in the present study (Ota, 1999).

In melanotropes, the cells immunoreactive to α-MSH antiserum had stronger immunoreactivity with reduced cell sizes in males. This result may indicate that these cells were in rest after the extensive secretion of α-MSH to provide nuptial color, since strong nuptial coloration was seen in FW males. Similar evidence in rainbow trout (Suzuki *et al*., 1997) supports this idea. Since the changes in α-MSH-ir and β-endorphin-ir cells were nearly the same, it is possible that β-endorphin-ir cells underwent extensive secretion prior to spawning and became

inactivated at the final stage of salmon life cycle. Hane *et al.* (1966) reported that activity of the adrenal cortex diminished progressively by the time of spawning in pacific salmon (*Oncorhynchus tshawytscha*). Getting together, we consider that POMC-derived hormone-producing cells are activated to secrete hormones prior to or during spawning migration in salmonid.

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