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Histological Changes in the Pituitary, Thyroid Gland and Gonads of the Fourspine Sculpin (*Cottus kazika*) during Downstream Migration

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ABSTRACT—The Fourspine sculpin (*Cottus kazika*) is a catadromous fish which is widely distributed in the rivers of Japan. The fish was used to examine the relationship between the migration behavior and hormonal control by studying the histological changes in the pituitary gland, thyroid gland and gonads during its downstream migration. By use of the immonocytochemical and histochemical techniques, 7 types of cells were identified in the pituitary gland namely; immunoreactive (ir)-PRL, GH, TSH, GTH, ACTH, MSH and SL cells. From among the first 4 types of the aforementioned cells, remarkable histological changes were observed in cells containing ir-GTH during the downstream migration. At this time also, the gonads were obsereved to be well developed, while the thyroid glands did not show clear changes morphologically. These results suggest that the gonadotropin regulates gonadal development in the Fourspine sculpin during downstream migration and possibly sex hormones synthesized by the gonads cause the downstream migration of this catadromous fish.

INTRODUCTION

Until recently a number of studies have been performed on migratory fishes, in particular to osmoregulation, smoltification, homing migration and migratory bahavior. In salmonid fish, it was suggested that pituitary hormones, thyroid hormone and sex hormones are closely related to migration [23]. Although physiological studies of salmonid fish are developed, there are many other groups of migratory fishes which are not investigated.

The family Cottidae accomplished distinctive adaptive radiation. In Japan, there are some kinds of freshwater cottid fish and they exhibit various types of ecological life-styles. *Trachidermus fasciatus* [35] and *Cottus kazika* [19] are catadromous fish. *C. hangiongensis* [11], *C. amblystomopsis* [9, 10] and small egg type of *C. pollux* [21] are amphidromous fish and *C. nozawae* [9, 10] and large egg type of *C. pollux* [21] have land-locked life-span. One of these freshwater cottid fish, Fourspine sculpin (*Cottus kazika*), known in Japan as Ayukake or Kamakiri is endemic species of Japan [22]. They inhabit middle river basin during spring to autumn, go down the river in later autumn, and spawn in the coastal sea area in winter [19]. Although these life formes are known, the physiological studies that will give on insight to the existence of the forms specifically the migratory behavior have not been reported.

In the present study, immunocytochemical and histochemical techniques were used for distinct the pituitary cells in the cottid fish pituitary. We aimed to analyze the histological changes of pituitary cells, thyroid gland and gonads in the cottid fish, Fourspine sculpin, during downstream migration and discussed to correlating changes of these endocrine organs with downstream migration.

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MATERIALS AND METHODS

Animals

Two groups of Fourspine sculpin (*Cottus kazika*) were used in this study. The first group (standard length 5.8–13.7 cm), settled in Warashina river, a tributary of Abe river located in Shizuoka prefecture was collected from July to September 1992 by casting- or landing-net. This group was referred here as the river group.

The second group (standard length 7.8–14.8 cm), called downstream migration group as the name implies, was collected from November to December 1992 from estuary of Abe river.

Tissue preparation

After capture, both male and female fish were weighed, and sacrificed by decapitation. The brain with a pituitary attached was removed, and fixed in Bouin-Hollande sublimate solution for about 24 hr. The fixed tissues were dehydrated through a series of solutions with increasing concentrations of ethanol, and embedded in paraffin. Serial sagittal sections were cut in 6 μm and mounted in sequential set. Thyroid glands and gonads were also removed, fixed in Bouin-Hollande sublimate solution, and embedded in paraffin. Serial sections were made at 6 μm and stained with periodic acid Schiff (PAS) stain or Hematoxylin-Eosin, respectively.

Immunocytochemical study

For immunocytochemical study, avidin-biotin peroxidase complex (ABC) method was adopted [15]. The following antisera obtained from rabbit were used: anti-chum salmon prolactin (chumPRL), anti-chum salmon growth hormone (chumGH) (both antisera were raised by Dr. H. Kawauchi, Kitasato University) [3, 24], anti-human thyroid stimulating hormone β-subunit (this antiserum was raised by Dr. S. Raiti, Natural Pituitary Agency)

(hTSHβ), anti-silvercarp gonadotropic hormone (scGTH) (this antiserum was raised by Dr. K. Aida and Dr. M. Kobayashi, University of Tokyo) [18], anti-coho salmon GTH-I β-subunit (cohoGTH-Iβ) and anti-coho salmon GTH-II β-subunit (cohoGTH-IIβ) (these antisera were raised by Dr. P. Swanson, University of Washington) [33] and anti α-melanophore stimulating hormone (αMSH) (this antiserum was raised by Dr. K. Wakabayashi, Gunma University).

In some experiments, antisera against chumPRL, chumGH and scGTH were preincubated with purified chum salmon PRL, chum salmon GH (kindly donated by Dr. H. Kawauchi, Kitasato University) and silvercarp GTH (kindly donated by Dr. K. Aida and Dr. M. Kobayashi, University of Tokyo) respectively. Incubation with these antigen abolished staining. Other sections were incubated in the absence of primary or secondary antisera which also abolished staining.

In addition to the immunocytochemical staining, some sections were stained histologically with aldehyde fuchsin (AF), PAS or lead hematoxylin (PbH).

Morphometric study

Several series of 10 or 20 successive sagittal sections of pituitary region were made. The first section of every series of successive sections was gathered and mounted on the same glass slide. Every 2nd to 10th (or 20th) section was mounted on the glass slide in the same manner. After immunostaining, total number of immunoreactive cells to each antiserum was calculated per animal under a light microscope. The number of ir-PRL, GH, TSH and GTH cells was counted from 3 midsagittal pituitary sections, and the means of the number of immunoreactive cells per section were calculated for each fish. Finally the data were analyzed statistically using the Mann-Whitney U test.

To analyze the thyroid function, measurements of epithelial height of the follicular cells were made. Average cell heights were determined for 10 follicles chosen at random from the thyroid glands of each animal. The Mann-Whitney U test was used for statistical analysis.

To examine the gonadal development, GSI (gonad somatic index: gonad weight$\times$100/ body weight) was calculated per animal.

RESULTS

Immunocytochemistry of pituitary

The pituitary gland of Fourspine sculpin consists of adenohypophysis and neurohypophysis. The adenohypophysis is clearly divided into rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). Immunocytochemical staining revealed that the major part of RPD was occupied by ir-PRL cells (reacted with anti-chumPRL) (Fig. 1A) and the minor part by ir-ACTH cells (reacted with anti-αMSH) located closely to the neurohypophysis (Fig. 1B). With respect to PPD, its dorsal part was occupied solely by ir-GH cells (reacted with anti-chumGH) (Fig. 1C), its dorsal-anterior part by ir-TSH cells (reacted with anti-hTSHβ) (Fig. 1D), and its ventral part by scattered ir-GTH cells (reacted with anti-scGTH), which also extended posteriorly to form

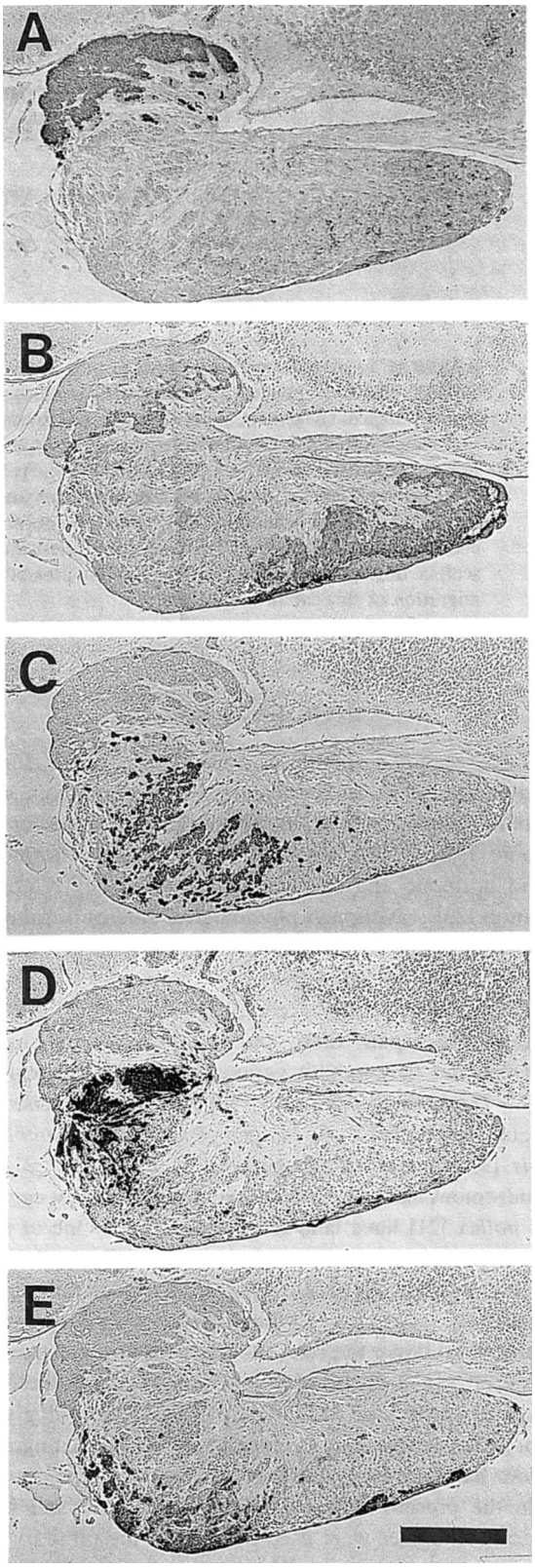

FIG. 1. Sagittal sections of Fourspine sculpin pituitaries. A) Immunostained using antiserum to chum salmon prolactin (chumPRL). B) Immunostained using antiserum to α-melanophore stimulating hormone (αMSH). C) Immunostained using antiserum to chum salmon growth hormone (chumGH). D) Immunostained using antiserum to human thyroid stimulating hormone β-subunit (hTSHβ). E) Immunostained using antiserum to silvercarp gonadotropic hormone (scGTH). Note the regional differences in the distribution of cells. Anterior is on the left. Bar indicates 200 μm.

TABLE 1. Immunocytochemical and histochemical staining of the adenohypophysis of Fourspine sculpin

Used antiserum and histochemical method	RPD		PPD			PI	
	PRL	ACTH	GH	TSH	GTH	MSH	PAS(+)
chumPRL	++	−	−	−	−	−	+
chumGH	−	−	++	−	−	−	−
hTSHβ	−	−	−	++	−	−	−
αMSH	−	++	−	−	−	++	−
scGTH	−	−	−	−	++	−	−
cohoGTHIβ	−	−	−	−	−	−	−
cohoGTHIIβ	−	−	−	−	+	−	−
AF	−	−	−	++	++	−	−
PAS	−	−	−	++	++	−	++
PbH	+	++	−	−	−	++	−

−, +, ++ negative, weak, and strong staining responces, respectively.

the external border of PI (Fig. 1E). This type of GTH cells was also reacted with anti-cohoGTH-IIβ weakly, and stained with PAS. With respect to PI, it was occupied largely by ir-MSH cells (reacted with anti-αMSH) and its border region adjacent to neurohypophysis was occupied by cells showing weak immunoreactivity to anti-chumPRL. Histologically, this last type of cells did not stain PbH, but selectively stained with PAS. These cells were assigned as ir-SL (somatolactin) cells. Table 1 and Figure 2 show the results of immunostaining and histochemical staining.

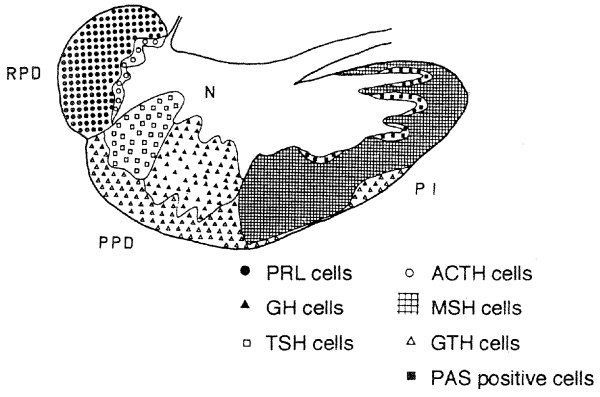

FIG. 2. Schematic diagram showing localization of pituitary cell types identified by immunocytochemical and histochemical methods. RPD, rostral pars distalis; PPD, proximal pars distalis; PI, pars intermedia; N, neurohypophysis.

Histological changes in pituitary cells

No histological changes of PRL cells and GH cells were demonstrated between the river and the downstream migration groups of sculpin. PRL cells were polygonal and had much cytoplasmic materials, while GH cells were round in shape and had clearly round nuclei. The number and the staining intensity of both cells were not different between two groups (Fig. 3A, B).

Although morphological changes of TSH cells were not demonstrated, the number of the TSH cells in the downstream migration group was slightly but significantly ($P<0.05$) greater than the number of cells in the river group (Fig. 3C). The number of the GTH cells in the downstream migration group was clearly and significantly ($P<0.05$) greater than the cells in the river group (Fig. 3D). The cell sizes of the GTH cells in the river group were small. On the other hand, the cell sizes of the GTH cells in the downstream migration group were enlarged and had abundant cytoplasmic material with large nuclei (Fig. 4).

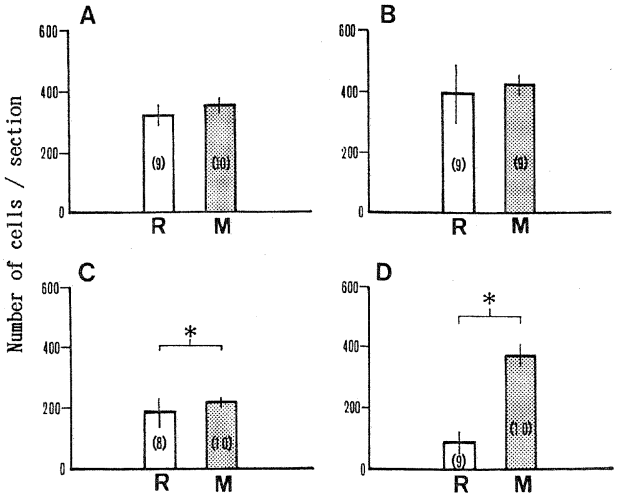

FIG. 3. The number of 4 types of pituitary cells in two groups of Fourspine sculpin. A) PRL cells; B) GH cells; C) TSH cells; D) GTH cells. The vertical bars represent the mean ± SE. R, river group; M, migration group. *Significant difference ($P<0.05$) between two groups. The number of animals is shown in parentheses.

Histological changes in thyroid gland

Like those of many other species of bony fishes, the thyroid gland of the Fourspine sculpin is a diffuse tissue consisting of follicles which are scattered over the connective tissue with rich vascularities along the anterior ventral aorta and its afferent branchial arteries.

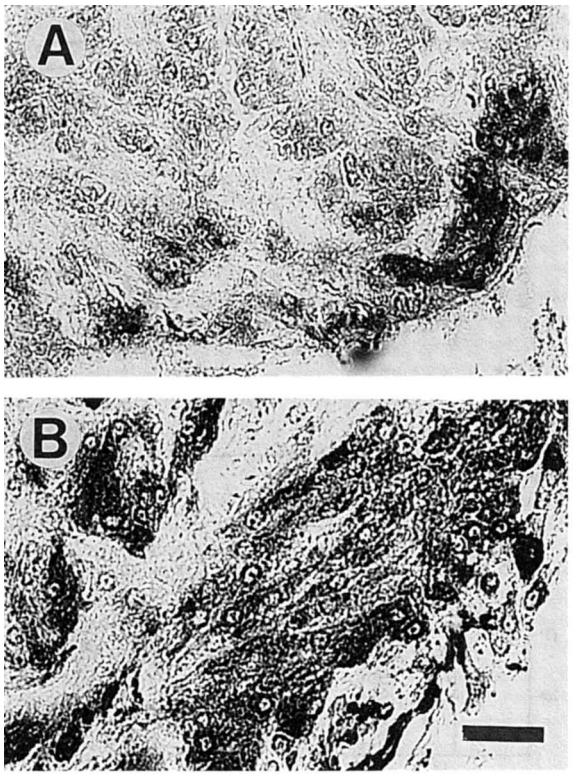

FIG. 4. Sagittal section of Fourspine sculpin pituitary immunostained with anti-scGTH. A) GTH cells of the river group of Fourspine sculpin. B) GTH cells of the downstream migration group of Fourspine sculpin. Bar indicates 20 μm.

In the river group, the structure of thyroid gland was different from each individual, and the range of cell height of the follicular epithelium was wide (approximately 7–30 μm). On the other hand, in the downstream migration group, cell heights of the follicular epithelium were relatively similar (approximately 17–21 μm). However, there was no significant difference in the height of the follicular epithelium

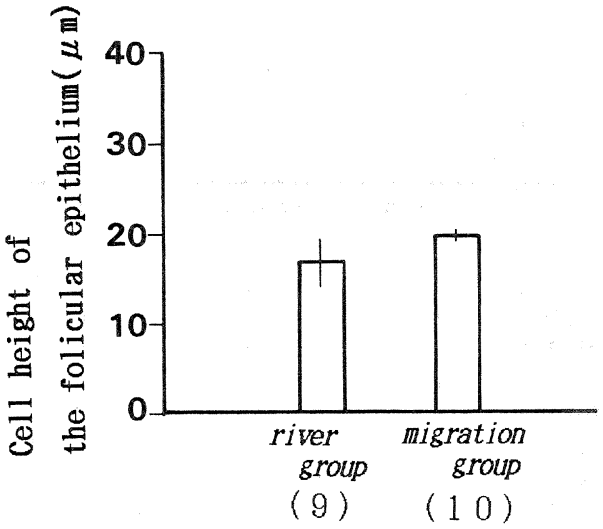

FIG. 5. The height of epithelial cells in thyroid gland in two groups of Fourspine sculpin. The vertical bars represent the mean ± SE. The number of animals is shown in parentheses.

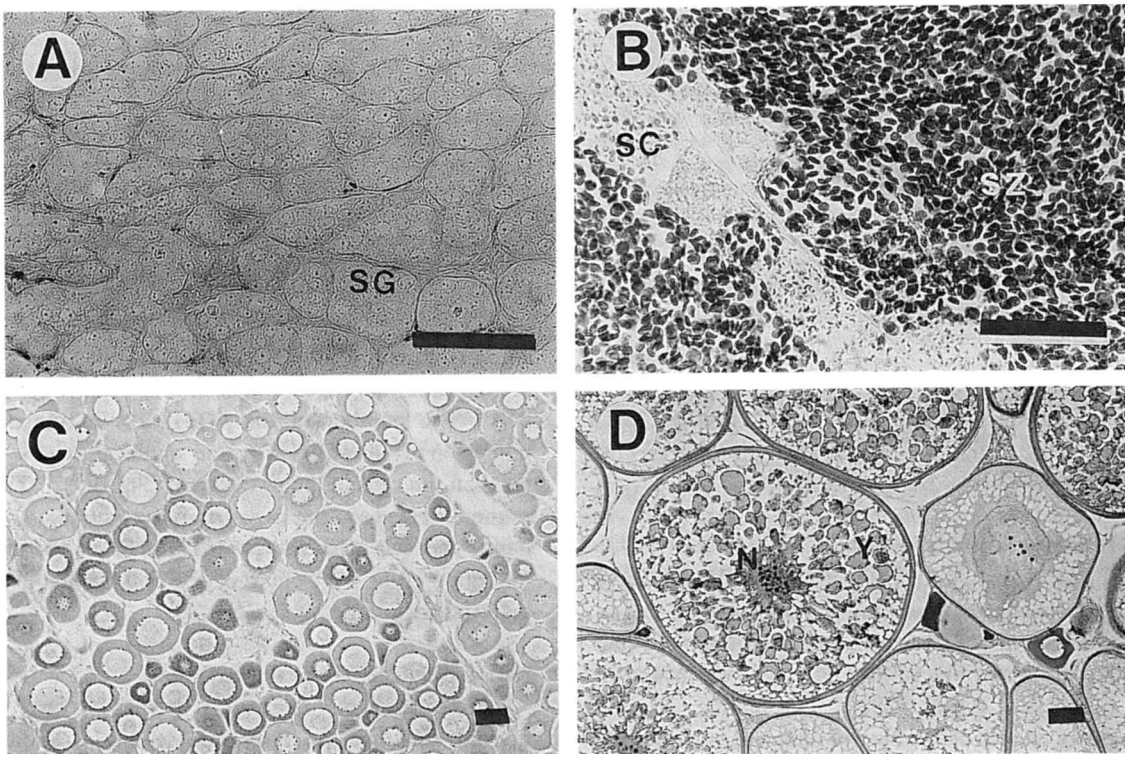

FIG. 6. Histological changes in gonads of two groups of Fourspine sculpin. A) The testis of the river group. B) The testis of the downstream migration group. C) The ovary of the river group. D) The ovary of the downstream migration group. Each symbols indicates: N, nucleus; SC, spermatocyte; SG, spermatogonium; SZ, spermatozoa; Y, yolk vesicle. Hematoxylin-eosin stain. Bars indicate 50 μm.

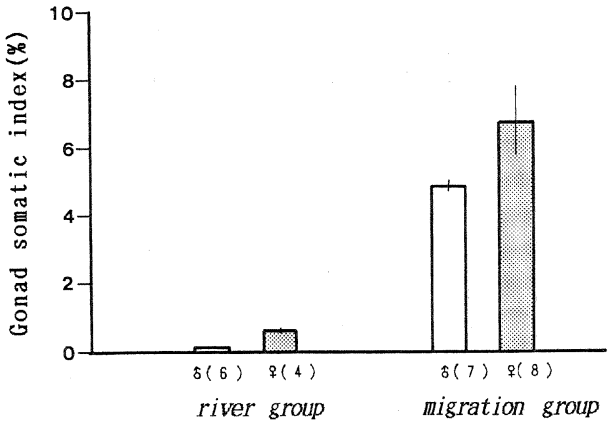

FIG. 7. Gonad somatic index (GSI) in two groups of Fourspine sculpin. The vertical bars represent the mean ± SE. The number of animals is shown in parentheses.

between the two groups (Fig. 5) and could not find the different condition of follicular colloid.

Histological changes in gonads

Histological examination showed that the testis in the river group had only spermatogonium (Fig. 6A), whereas the testis in the downstream migration group had many sperms and various stages of spermatocytes (Fig. 6B). The ovary in the river group had immature eggs at the peripheral nucleolus period (Fig. 6C). On the other hand, the ovary in the downstream migration group had the eggs characterized by many yolk vesicles and at the stage of vitellogenesis (Fig. 6D).

The GSI also showed the gonadal development. The fish in the river group were immature and indicated low GSI, whereas the fish in the downstream migration group were maturing and indicated high GSI (Fig. 7).

DISCUSSION

In the present study, pituitary gland, thyroid gland and gonads were compared between Fourspine sculpin settled in river and those of migrating downstream.

Using immunocytochemical method, the localization of different pituitary cells was studied. PRL, GH, TSH, GTH, ACTH and MSH cells were reacted to antibody against the hormone that each of these cells synthesized. Furthermore, a second type of PI cells was demonstrated here. The PI cells that reacted to anti-chumPRL could be identified to be somatolactin (SL) cells. According to Ono *et al.* [27] the amino acid sequence of the somatolactin was similar to those of PRL and GH. It has been shown that the distribution pattern of the PI cells that immunoreacted to anti-chumPRL and stained by PAS agreed with the distribution of SL cells in other teleosts [29]. The distribution of these 7 cell types of pituitary cells in the Fourspine sculpin agreed well with those of other teleosts [1, 7, 41].

In this study, only one type of GTH cells was observed. In salmonid fish, the two gonadotropins which were called GTH-I and GTH-II were produced by two distinct cells and GTH-I induced vitellogenesis while glycoprotein GTH-II induced egg maturation [25, 26, 31, 32]. Although it is uncertain that the Fourspine sculpin has two gonadotropins or not, the immunoreactive GTH cells in the Fourspine sculpin may be GTH-II cells because 1) it was reacted anti-cohoGTH-IIβ and stained by PAS, and 2) the purified silvercarp GTH (the antigen of the anti-silvercarp GTH) could be GTH-II since it is a glycoprotein GTH [17] and the ovulatory surge of this type of GTH was observed [18].

Morphological changes in PRL, GH, TSH and GTH cells were studied. It is well known that PRL plays a role in freshwater adaptation [20], while GH plays a role in seawater adaptation of salmonid fish [4, 6]. The estuary of Abe river has little influence by seawater thus the fish is not affected by a change in environmental salinity. Consequently, it may be said that PRL and GH cells had not been working to adapt both groups of the Fourspine sculpin to seawater. In newts, it is known that PRL causes the water drive behavior [5]. However, the PRL and GH cells did not show morphological changes in the Fourspine sculpin during downstream migration. It may be posturated that PRL and GH in the Fourspine sculpin do not play important roles in downstream behavior and also in gonadal maturation.

Although TSH cells significantly increased during downstream migration, no morphological changes in TSH cells were observed. Moreover, the thyroid gland in downstream migration group had not much more activity than the thyroid gland in river group because the cell height of the epithelial cells of the thyroid gland was not significantly different between the two groups. On the other hand, histological changes of thyroid gland were observed in some other fishes, ayu [14], eels [16] and ice goby [34], during migration period. In salmonid fish, it was reported that smoltification was caused by thyroid hormone during downstream migration of juvenile [2]. Moreover, it was suggested that thyroid hormone was closely related to migration behavior in coho salmon (*Oncorhynchus kisutch*), masu salmon (*O. masou*) and amago salmon (*O. rhodurus*) since thyroxine (T_4) surge occurred in fish just before the downstream migration [13, 39, 40]. Although thyroid hormone may influence smoltification and downstream migration in the salmonid fish, it is not known whether T_4 induces the migration behavior in the Fourspine sculpin.

In winter time, Fourspine sculpin goes down from upper basin of the river to coast to spawn during in winter [19]. In the present study, the fish caught in estuary in this time had well developed gonads and activated GTH cells. Sasaki [30] reported that the Fourspine sculpin in upper basin on December had immature ovary. The other freshwater cottid fish, *Cottus hangiongensis,* lives in the middle to lower basin of river, and they go down the lower basin in spawning period [11]. Adult male of *C. hangiongensis* having immature testis during spawning period was reported to stay in upper basin away from lower basin of spawning place [8, 36]. These suggest that the gonadal maturation is associated with down-

stream migration of freshwater sculpins. Although it is uncertain that the relationships between gonadal maturation and migration bahavior of diadromous fishes, it was reported that sex hormones modified the spontaneous and evoked bulbar electrical activity in gold fish [28], and serum estradiol and testosterone increased during the parr-smolt transformation of masu salmon (*Oncorhynchus masou*) [38]. Therefore, it may be possible that sex hormones are one of the most important factor to start the migration behavior. It remains for further study to determine whether the sex hormonal contents are changed during downstream migration in Fourspine sculpin.

In the family Cottidae, 52 genera of cottid fish are live in the sea while only the 4 genera (*Trachidermus, Cottus, Cottocomephorus* and *Myxocephalus*) live in freshwater [37]. Therefore, in the comparison of the life-styles of *Cottus* species, these freshwater *Cottus* species may originate from a group of marine sculpin [12]. Thus it may also be conjectured that the habit of the spawning of Fourspine sculpin in seawater may be a primitive character, while that of spawning in freshwater is a recently one. Having acquired the capability of spawning in freshwater, these fish, the land-locked ones, do not need migrating behavior induced by gonadal maturation. Thus, the neural system which controls the spawning migration would be linked the gonadal maturation through the sex hormone in catadromous fish, while is not linked or is absent in the land-locked fish.

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