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# Histological Changes in the Organs and Tissues of the Gobiid Fishes throughout the Life-Span—II The Hypophyseal Target Organs of the Ice-Goby, *Leucopsarion petersi* Hilgendorf

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**Abstract** Previously, Tamura and Honma traced the changes occurred in the histology of the hypothalamo-hypophyseal neurosecretory system of a small goby, *Leucopsarion petersi*, based on the materials collected in selected stages of their habitat from sea water to spawning ground in streams. The present study, with objectives to expand knowledge as to their mode of annual life especially on reproduction physiology, deals with histological changes traced along their migratory and reproductive activities in the thyroid, interrenal gland (adrenal cortical tissue) and gonads among the target organs of hypophysis. The thyroid appeared inactive in the fish in their sea life; its activity grew higher in those ascending stream in early season of their migration, and it lowered as season proceeded and showed the minimum in the latest migrants; all these facts suggested that the organ is not concerned with osmoregulatory function. The thyroid activity reached highest on two sexes at the spawning site though that of females still surpassed that of males and, as expected, the exhaustion of the gland activity was more accelerated in females. The interrenal gland showed a slight indication of regressive degeneration in the fish at the spawning site. The ovarian eggs, categorized synchronous, developed rapidly during the course of their ascending migration to the spawning ground. The spent ovary showed a number of ovulation scars, but ultimate corpus luteum was not found even in the fishes just died. The tubular testis of the present species lacks accessory gland such as seminal duct. The development of spermatozoa, asynchronous, reached the maximum in the fish arriving spawning site. The so-called relict spermatozoa were found in a considerable amount remaining in testicular lumen of the fish died after reproductive activity.

## Introduction

As stated in Tamura and Honma (1969), the ice-goby, an annual fish dwelling entire coast of Japanese Islands, ascends the stream for spawning in spring, and dies several days after a single spawning. The hatched larva descends the river to the sea in a few days. The life-history of this small goby in the sea has not been known up to the present time, but Yabe (1940) made an account of its behavior before and after the breeding in the estuaries of Korea. According to Kitahara (1904), this goby having an obvious sexual dimorphism lives in the shallow water of the sea, and enters clear streams with gravelly bottom where the fish spawns. But the fish never goes up the stream to a great distance and remains within 5 or 6 km of the mouth of a river. And its eggs are deposited on relatively larger stones burried in sands and pebbles.

The histological changes found in some of the organs and tissues accompanying sexual maturation and senility of the salmon and salmon-like fishes have been extensively investigated by Robertson and Wexler (1959, 1960, 1962), Robertson et al. (1961), Honma (1959, 1960, 1968), Honma and Suzuki (1968) and Honma and Tamura (1963, 1965, 1968). Honma (1966) also described a histologically pronounced changes in the organs of the mature catadromous eels taken from the

deep sea, and compared them with those of the freshwater phase. Tamura and Honma (1969) reported the activity of the hypothalamo-hypophysial neurosecretory system of the ice-goby having a peculiar pattern of the system. In order to compare other anadromous and catadromous fishes, histological pictures of some of the target organs of hypophysis of the ice-goby, such as the thyroid gland, interrenal gland (adrenal cortical tissue), and gonads, were observed microscopically with a special attention of post-spawning death of the fish.

## Material and methods

All of the specimens used in the investigation of the hypothalamo-hypophyseal system (Tamura and Honma, 1969) were also used in the present study. The specimens were collected in each period of their upstream migration up to post-spawning, and the specimens in their sea life were taken from fishes caught by beam-trawl net at a depth of about 60 meters off the coast of Kamo-town, Yamagata Prefecture. The collecting places therefore extend from Niigata Prefecture to Yamagata Prefecture (38°12′–50′N, 139°26′–30′E). In addition to the adult form, several number of larval fish caught from the neighborhood of spawning beds were also studied for the ontogenetic observation.

The routine histological procedure was adopted for fixation, dehydration, embedding and staining, identical with that in those techniques adopted in the previous paper (Tamura and Honma, 1969).

In order to follow the thyroid function, changes in the diameter of the follicles and the height of the follicular epithelial cells of the gland of the specimens were measured and plotted (Fig. 1). Further, the position and feature of the nucleus of the epithelial cell and the condition of colloidal substance accumulated in the lumen were also observed.

## Results

### Thyroid gland

The thyroid gland of the ice-goby, like those of many other species of bony fishes, is diffuse tissue consisting of the follicles which are scattered over the connective tissue with rich vascularities along the anterior ventral aorta and its afferent branchial arteries.

In the gland of the fish caught in the sea in March, an inactive figure was seen, i.e., the diameter of the follicles attains 47–67 $\mu$, and the lumina each surrounded by flat epithelium (4–6 $\mu$) are filled with much colloid (Fig. 1, Pl. 1–A). A pronounced change was observed in the gland of different shoals of fish due to their time of ascending the river; the period of upstream migration lasting from middle April to late May (Fig. 1). In the earliest runners of upstream migrants in middle April, the size of the follicles is large (64–93 $\mu$ in diameter), and the height of epithelium is nearly twice as high as that in the preceding season (8.6–11.8 $\mu$). Many vacuoles are found in the periphery of the colloid in the lumen, so that the colloid shows a wavy margin. These features will indicate a normal activity or a slightly hyperfunctioning state of the thyroid gland (Pl. 1–B). By the later in May, the gland of reinforcements becomes gradually more inactive during the period of the highest migratory activity toward the end of upstream movement. Early in May, the size of follicles reduces considerably, 58–82 $\mu$ in diamter, and the epithelium consists of cubic cells, 6.8–7.3 $\mu$ in height. Decrease in the height of epithelial cells of the migrants continues successively untill the end of May. Simultaneously, gradual diminution in the amount and viscosity of the colloid is detected with its marked wavy margin. This phase seems to represent a state of hormone release from the gland (Pl. 1–C).

Though the diminution in the size of follicles proceeds still more (30.9–48.6 $\mu$) in the fish obtained from the spawning sites late

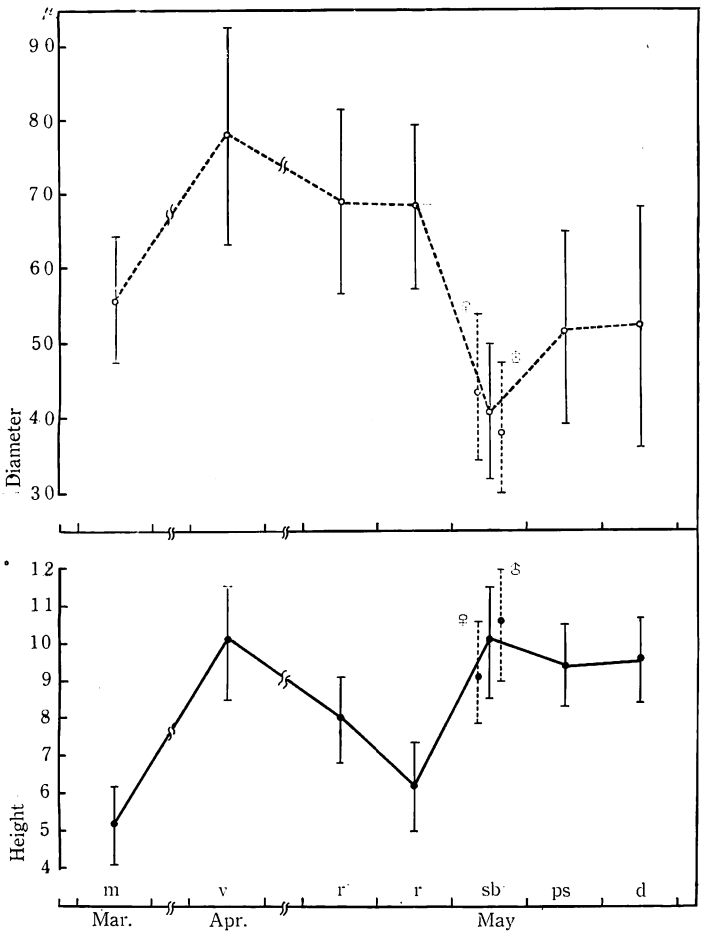


Fig. 1. Follicular size (upper figure) and the height of epithelial cell in thyroid gland (lower) of *Leucopsarion petersi* measured on adult fishes collected, with advance of season, in six life phases; sea life (m), earlier migrants in rivers (v), later migrants (r), spawners (sb), spent (ps) and the dead after spawning (d). Note the sexual difference in both diameter and height on the fishes in their reproductive activity.

in May, the epithelial cells notably regain their height. It is interesting to see the sexual differences of the thyroid function; in spite of smaller size of the follicles in the male fish, the height of epithelial cells is greater than in the female one; 9.0–12.7 $\mu$ in male and 7.9–9.1 $\mu$ in female. There are many vacuoles in the cytoplasm of the female fish, indicating a severe exhaustion of the gland (Pl. 1–E). On the contrary, the gland of the male yet retains a state of a marked hyperactivity, and, as compared with that in the female, the rate of degeneration is less significant. In the fish immediately before the death, the indication of regression and collapse of the cells is more significant than in the preceding stage, and the increase in number and size of the vacuoles is prominent (Pl. 1–F).

**Interrenal gland (adrenal cortical tissue)**

The interrenal gland of the ice-goby is observed as several patches occurring in the anterior portion of cardinal vein approximating dorsally to the heart. The amount of the tissue of this gland is very scanty.

Unfortunately, no histological detail was

available on the material collected from the sea. The specimens caught throughout the period of upstream migration showed the gland with rather large, round cells (10–12 $\mu$) including the round nuclei. An increase in the number of these cells is not well noted compared with that seen in preceding period, and the tissue consisting of one or two layers of these cells surrounds the cardinal vein (Pl. 1–G). In the specimens taken from the spawning bed and postspawning fish before death, a slight increase in the number of the cells is detected. However, the shape of cells and nuclei is ovoid to elongate ovoid, and a slight decrease in the size of the cells is seen measuring 6 $\mu$ in shorter and 8 $\mu$ in longer axis. These figures seem to indicate a certain state of hyperplasia and further regression of the gland.

**Gonads**

In the material of adult fish obtained in the sea, the development of ovarian eggs reaches already an early stage of vitellogenesis, i. e., the yolk vesicle to globule stages with a still flat follicular epithelium and thecal membrane (Pl. 1–H). During the period of upstream migration, the yolk formation of the egg proceeds greatly accompanying a disappearance of the nucleus (=germinal vesicle). It seems that this state is equivalent to the migratory nucleus to prematuration stages, and the diameter of the egg attains to 350–400 $\mu$ (Pl. 2–J). As there is no marked difference in the rate of growth of the eggs contained in the ovary of the ice-goby, the developmental process of eggs seems nearly synchronous.

Due to rapid maturation, the eggs of the fish found in the spawning beds attain to the fully ripe stages with a simultaneous increase of the thickness of follicular membrane. A micropile is now visible in each egg without difficulty (Pl. 2–K). After oviposition, the ovary of the spent fish is occupied with a number of ovulation scars and a small number of eggs which are, in the perinucleolus to yolk vesicle stages, under the process of regression (Pl. 2–L). Further, ultimate corpora lutea and the oogonia and/or young oocytes which might be the source of functional eggs of next breeding season are absent.

It is interesting that the testis of the ice-goby is a single tubule without any accessory gland and lacks seminal vesicle, a characteristics found in many other species of gobiid fishes (Egami, 1960). In the specimens at the time of onset of anadromous migration and those in the route of migration, the central part of the testis is filled with innumerable number of spermatids, and peripheral region contained secondary spermatocytes. Then, the various stages of the cells under the secondary maturation division are found in this peripheral region (pl. 2–M). Following this, the formatin of spermatids and the further spermiogenesis take place rapidly, and, in the testis of the fish obtained from the spawning bed abundant spermatozoa are detectable. Further, the peripheral region of the testis in these fish yet contains the spermatids (Pl. 2–N). Even after the onset of ejection of sperm, a considerable amount of the spermatozoa, though a decrease in number of spermatids is recognizable. Considering the feature of spent testis mentioned above, the ejection of sperm in the mature ice-goby is believed asynchronous, and occurs in an extended duration, although all males die soon after the end of breeding (Pl. 2–P).

## Discussion

Death promised after the first spawning found in several species of both anadromous and catadromous fishes offers an interesting problem concerning the leading factors which determine the life-span of the fish. Since such phenomenon is unknown among mammals and seen relatively rare in fishes, a number of investigations were undertaken histologically and physiologically to disclose the nature of the postspawning death of Pacific salmon (*Oncorhynchus* spp.), the Ayu

(*Plecoglossus altivelis*), and Japanese eel (*Anguilla japonica*), and so forth (Robertson and Wexler, 1959, 1960, 1962; Robertson et al., 1961; Honma, 1959, 1960, 1968; Honma and Suzuki, 1968; Honma and Tamura, 1963, 1965, 1968; McBride et al., 1965). In comparison with those fishes mentioned above, the present ice-goby is believed suitable for histological examination, because material can be obtained more easily in most part of their life-span. Further, the present species presents peculiarities in the organs, such as hypothalamo-hypophyseal system (Tamura and Honma, 1969), excretory organ and its associate glands, and hemopoietic organs, and so forth (Tamura and Honma, in press).

It has been assumed that increased thyroid activity in fishes plays an important role for their oriented locomotory movements, such as migration and regulation of water and salt metabolism (Fontaine, 1954; Baggerman, 1957; Hickman, 1959; Woodhead, 1959a, b; Honma, 1959; Honma and Tamura, 1963). It is also reasonable to expect that thyroid hormone might exert various abilities of membrane system of fishes. Many factors influencing fish migration as well as thyroid hormone have extensively been reviewed by Koch (1968). One of the other evidences of the thyroid function concerns the sexual maturation accompanying reproductive phase or breeding season (Barrington, 1954; Baker-Cohen, 1961; Woodhead, 1966). From the evidences found in the two strains of the Ayu, *Plecoglossus altivelis*, (Honma and Tamura, 1963) and, in particular, the ice-goby, *Leucopsarion petersi*, described in this paper, the thyroid activity does not appear to associate with osmoregulation, and the true (or even adjunctive) function of the gland during the periods of fish migration is hardly determined under histological examination (Honma, 1968).

In addition, the sexual dimorphism is found in the histological pictures of the gland in the ice-goby; the degree of exhaustion in the gland of female fish is severer than in that of the male. This difference might be resulted from the difference of the post-spawning behavior between two sexes in the ice-goby; male fish stays on the stream bed and protects the deposited eggs for a time, but female succumbs soon after the deposition of eggs.

A pronounced hyperadrenocorticism both in histologically hyperplastic interrenal gland and in physiologically elevated plasma concentration of 17-hydroxycorticosteroids was found in the migratory spawning Pacific salmon, steelhead trout under the starvation or limited irregular feeding, two strains of the Ayu, and catadromous eel under the sexual maturation, and so forth (Robertson and Wexler, 1959, 1960, 1962; Oguri, 1960; Olivereau, 1960; Honma, 1960, 1966; Honma and Tamura, 1963). Hence, it is very natural to expect that the interrenal gland of the ice-goby in spawning would reveal a hyperplastic condition, but no remarkable changes, whether hyperactive or degenerative, were encountered in the specimens tested. In our knowledge there is one report on the guppy, *Lebistes reticulatus*, finding the increasing change along with age of the interrenal gland (Woodhead and Ellet, 1967). These authors diagnosed the changes as a process of regression with increased granulation due to picnosis of the cell, and considered that the aging of the guppy would follow a different course from that taken by the Pacific salmon. If this interpretation is right, the result obtained in the ice-goby might be in accordance with that of the guppy. Therefore, much more evidences in favor of this line is wanted in many other species with life pattern different from that of salmon and salmon-like fishes.

Many investigations have been carried out to elucidate the oogenesis (and/or spermatogenesis), vitellogenesis (spermiogenesis), and ovarian (testicular) cycles, and the relationships between gonadal maturation and the other endocrine function, in particular, the hypophyseal function (Ball, 1960), although patterns of ovarian and testicular activities