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Changes in the Thyroid Gland Associated with the Diadromous Migration of the Threespine Stickleback, *Gasterosteus aculeatus**

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Abstract The role of the thyroid gland of the diadromous threespine stickleback, *Gasterosteus aculeatus*, was studied in relation to the life history of the fish. The activity of the gland is high at the earlier period of ascending the river, while the activity is low in the sea. In the spawning season, an indication of exhaustion of the gland is seen, and the further regressive change occurs in the postspawning spent fish. The thyroid of juvenile fish caught near the spawning bed reveals a comparatively mild state. An inactive phase is obtained in the gland of the juvenile fish transferred into the sea water.

Mild state of the gland activity is secured in the materials of juvenile, adult and spent landlocked form, collected from May to October.

In the specimen reared in the freshwater aquarium for a long time (15 months), the gland expands greatly, showing some pathologic conditions, such as hyperplasia, hypertrophy and rich vascularities.

A considerably remarkable correlation between thyroid hormone and migratory behavior in both chondrichthyan and osteichthyan fishes has been presented by several investigators (von Hagen, 1936; Olivereau, 1954; Honma, 1959a; Woodhead, 1959a, 1959b; Honma and Tamura, 1963; Woodhead, 1966; Tamura and Honma, 1970; etc.). Using the European threespine stickleback, Baggerman (1957) and Koch (1968) have extensively investigated the thyroid function in association with the downstream or upstream migration and salinity preference. However, no report dealing with the Japanese stickleback was encountered. Therefore, histological study was performed to clarify the seasonal changes of thyroid activities of both marine and landlocked types of *trachrus* form. Further, to estimate one of the causal factors or mechanisms on the formation of landlocked type, the marine type propagated in the fresh water for a considerably long time was examined.

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Material and methods

A number of individuals of larval and adult threespine stickleback, *Gasterosteus aculeatus* L., collected from several places of Niigata and Fukushima Prefectures were used in this study. Details of most of the present specimens have been described in the previous paper (Honma et al., 1976). The marine type was obtained in the lower reaches of the Shinano River and also the Agano River located on Niigata City, Lake Kamo-ko (a salt lake in Sado Island), and 3 miles off the coast of the Sado Marine Biological Station of Niigata University. On the other hand, the landlocked type was collected in the upper reaches of the Agano River Basin near Kitakata City. The collecting places and their surroundings are illustrated (Fig. 1). The period of collection extended from February 1973 to October 1975.

In addition to these wild-caught specimens, the juvenile fish which were collected near the spawning bed or propagated in the laboratory aquarium were kept in 1/2 dilute sea water for 3 days, and then transferred into sea water. The fish were killed at 10, 23 and 38 days after acclimation. Each group

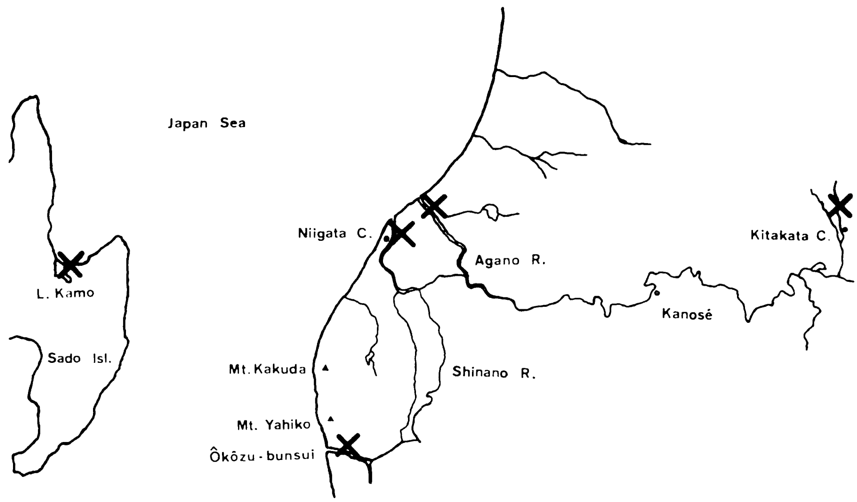


Fig. 1. Map of the collecting sites in Niigata area and its vicinity including Sado Island.

was consisted of 3 to 6 fish. Moreover, to make comparison with sea water specimens, a number of marine type fish hatched out were kept in a freshwater aquarium at about 16°C and killed 15 months later.

The thyroid gland together with its nearby structures removed was immersed in Bouin-Holland-sublimate solution, embedded in paraplast following a series of dehydration and cut serially 6 to 7μ thick in sagittal and cross directions. The sections were stained by various methods, such as Delafield's hematoxylin-eosin, azan trichrome and periodic acid Schiff (PAS) reaction counterstained by light green and orange G.

Three to 10 fish were selected for morphometric analysis from every time of fixation. The size — 1/2 (longer axis+shorter axis) — of 20 follicles of each fish was measured. Next, the height of the epithelial cell in three different regions of each follicle was also measured in these 20 follicles. Thus, the average value was calculated for the fish at all developmental stages.

Results

The thyroid gland of the threespine stickleback is located diffusely on the dorsal surface of the ventral aorta from the bifurcation of the first branchial artery to the junction of the second and third branchial arteries. The gland consists roughly of both anterior and posterior lobes.

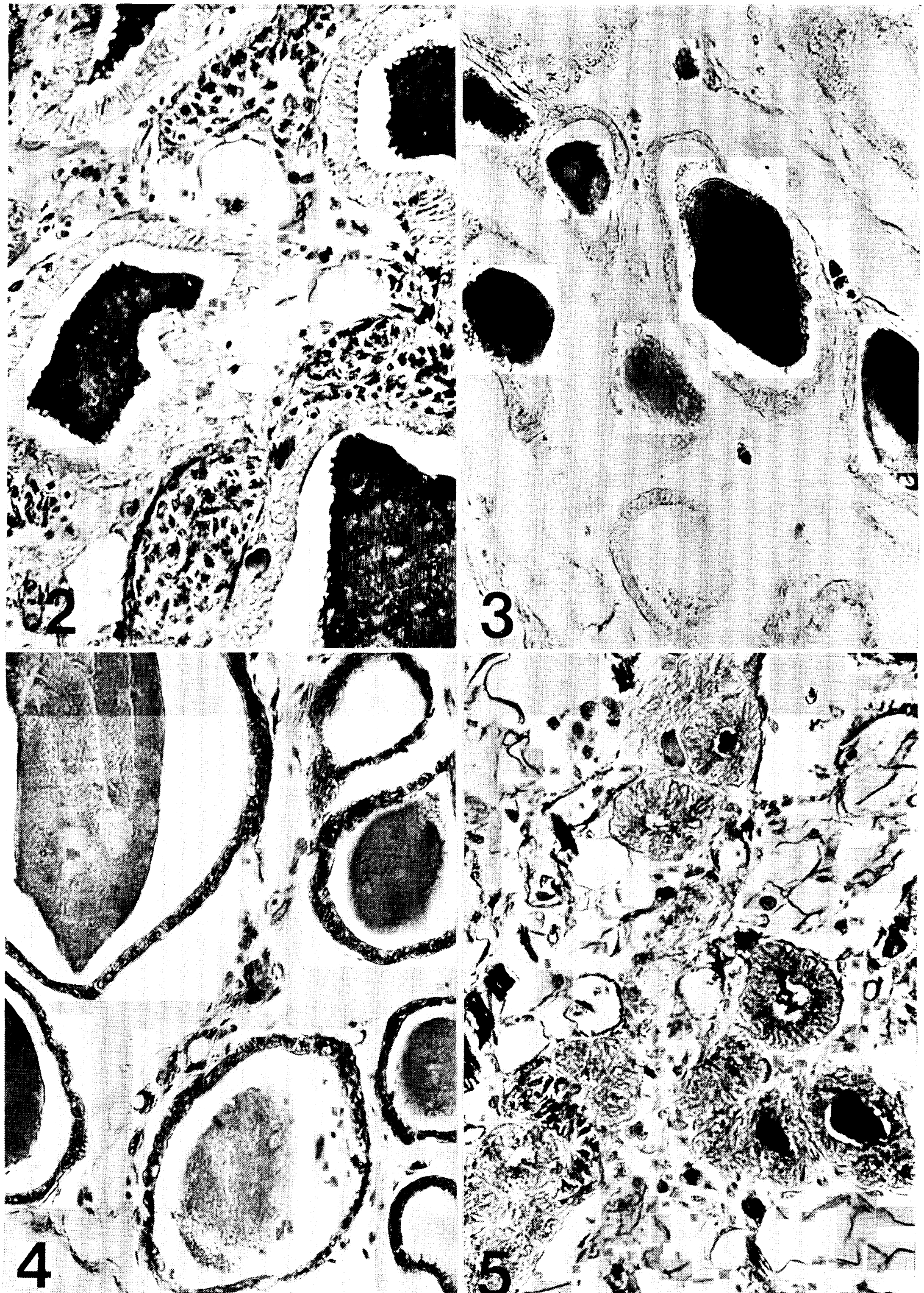
The highest level of the gland activity is detected in the earliest migrant caught at the river mouth in February (Fig. 2). The follicle with remarkably irregular outline is small, 45 to 75μ in diameter. The epithelial cell with a round nucleus is of high column, measuring 8.5 to 9.1μ. The amount of the

Fig. 2. Thyroid of the threespine stickleback just entered in upstream migration as vanguard of the shoal in February. The follicle reveals an active phase with columnar epithelial cells and heterogeneous colloid. Note well-developed vascularization around the follicle. Hematoxylin-eosin stain. ×450.

Fig. 3. Thyroid of the fish caught in the offshore water of the Japan Sea in April. A remarkable hypofunctioning state is detected. The ovoid follicle consisted of a flat epithelium maintains a spacious lumen. Azan stain. ×200.

Fig. 4. Thyroid of the fish taken from Lake Kamo-ko, a salt lake in Sado Island in May. Nearly identical picture as in Fig. 3 is encountered. H-e stain. ×450.

Fig. 5. Hyperfunctioning thyroid of the spawning fish collected at the breeding place in May. Regressive change is seen in this picture. H-e stain. ×450.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colloid in the lumen is also small, and the intensity of affinity for dyes is variable. The colloid detached from the epithelium shows a wavy margin with a number of vacuoles. Moreover, well-developed vascularization is noticed around the follicles. On the contrary, the lowest activity is seen in the specimens both from the sea and the salt lake before entering the river (Figs. 3, 4). Some of the ovoid-shaped follicles surrounded with a very flat epithelium attain twice the size of the specimen of the earliest migrant. The epithelium consists of flat to cuboidal cells, and its height is about a half of the earliest migrant. There is a comparatively dense and smooth colloid in the lumen. In April, however, the epithelium reduces its height accompanying somewhat a degenerative change.

At the beginning of the breeding season, the thyroid consists of small follicles with hypertrophic epithelium, and the gland of the specimen collected at the spawning place from May to June indicates a variable degree of exhaustion and regression (Fig. 5). But, demonstration of the nucleus in the epithelial cell is difficult due to a condensation and complication of cytoplasm, which sometimes indicates a needle-shaped condition. The narrow lumen contains a small amounts of colloid or no colloid. A relative increase in the amount of connective tissue is noticed in the postspawning spent fish.

The thyroid of the juvenile fish caught near the spawning place shows a rather mild activity : small follicles consists of cubic cells with round nuclei, and the lumen contains a dense, smooth and homogeneous colloid (Fig. 6). The pictures of both aquarium propagated and wild-caught juvenile fish transferred into sea water under the laboratory condition are closely similar to those of the specimen from and near the spawning place.

There is no noticeable seasonal change in the thyroid of the both juvenile and adult landlocked fish, although the materials examined were obtained from May to October. Even in the breeding season, the size of the follicle of the adult landlocked fish is large and the height of the epithelium consisting of cubic cells is low (Fig. 7). The colloid in the lumen is a smooth and homogeneous nature without wavy margin, and its amount is a comparatively large. There is no essential difference between the histologic pictures of the juvenile and adult specimens both collected in summer season.

These changes in the diameter of the follicles and the height of the follicular epithelial cells of the thyroid gland measured about every specimen were plotted and illustrated in Fig. 10.

The juvenile fish maintained for several months in a freshwater aquarium after the period of its seaward migration indicates a noticeable expansion in the region of lower jaw accompanying somewhat an exophthalmic condition, silverication of body color and dwarf body. After being kept for more than 15 months in such a condition, this gular expansion is much enlarged and is visible as a large swelling. Histologically, this thyroid swelling is well defined from other structures by a membranous loose connective tissue covering the outer layer of the gland (Fig. 8). It is easy to notice well-developed vascularization in the gland. Some of the canals are

Fig. 6. Thyroid of the juvenile fish near the breeding place in June. The follicle in mild condition consists of cubic epithelial cells. H-e stain. ×450.

Fig. 7. Thyroid of the landlocked fish caught in the upper reaches of Agano River, Aizu District, in August. The follicle consisted of cubic epithelial cells contains a lot of smooth colloid. H-e stain. ×450.

Fig. 8. Thyroid of the fish kept in freshwater aquarium for about 15 months after the period of its seaward migration. Note an extreme expansion of the gland with a remarkable proliferation of epithelial components and vascular canals. This condition is diagnosed as the adenomatous goiter. H-e stain. ×20.

Fig. 9. Enlarged view of the adenomatous goiter of the thyroid. Note that the colloid is almost absent in the narrow lumen, and the epithelium consisting of the high columnar cells reveals a long cord. H-e stain. ×450.


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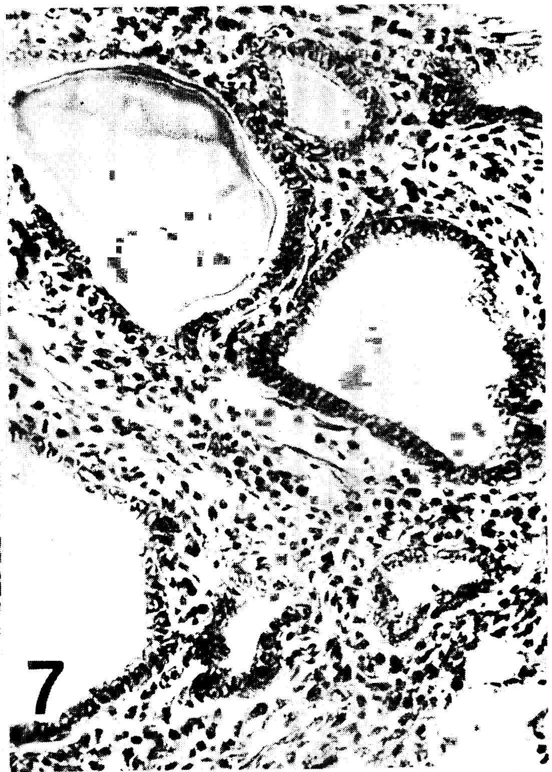
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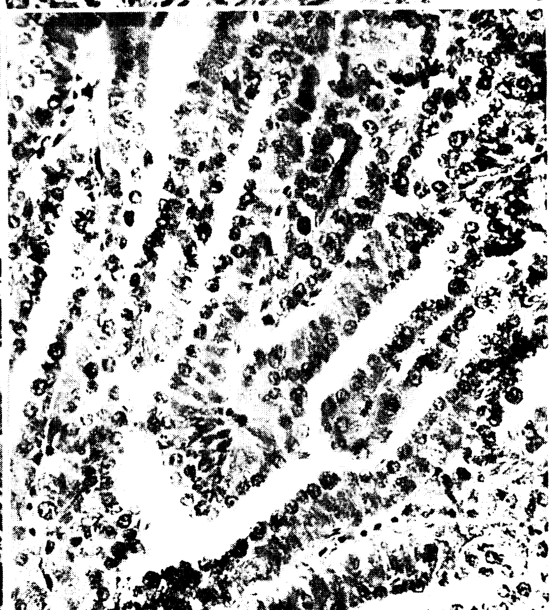
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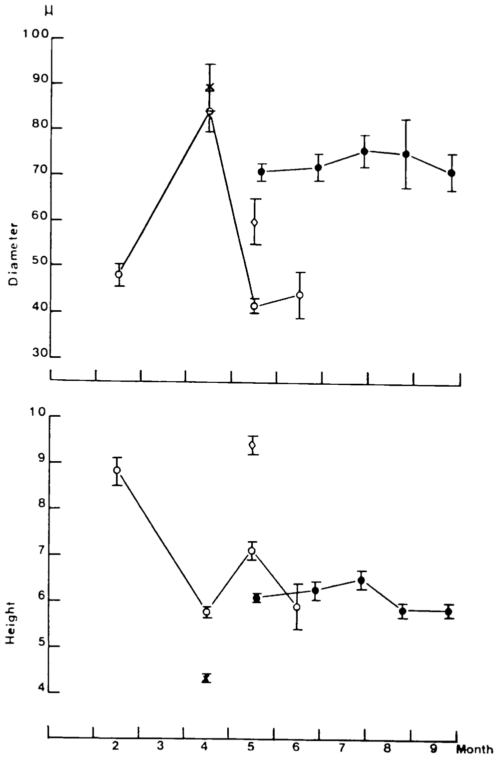

Fig. 10. Graph showing the follicular size (upper figure) and the height of epithelial cell in the thyroid gland (lower) of threespine stickleback, *Gasterosteus aculeatus* collected at different sites and months. ○ marine type, ● landlocked type, × specimen from Lake Kamo-ko, ◇ specimen from Ōkōzu-Bunsui canal just at the time of ascending the river.

dilated remarkably with a dense blood. Many of the follicular epithelium consisting of the high columnar cells show papillary growth (Fig. 9). The colloid is almost absent in the lumen, but the PAS positive colloid droplets are demonstrated in the region of free surface of the epithelial cell. The round nucleus is located near the free surface of the cell (Fig. 9). In some places of this dilated gland, there are found the large colloid spherules aggregated as a mass. These features described above are considered to be the adenomatous goiter of the thyroid.

Discussion

There are considerably much arguments on the possible role of the thyroid gland in fishes. Using the eel and starry flounder, the regulative function of the gland in salt and water balance was suggested by Fontaine (1956) and Hickman (1959). A certain parallelism of the function of the thyroid and reproduction was also intimated by Barrington (1954), Baker-Cohen (1961), Woodhead (1966) and Sage (1973). A possible relationship between the thyroid activity and migratory (or locomotory) behavior of the fish was demonstrated in the upstream fishes, and histologically hyperfunctioning evidence was secured in the elver (von Hagen, 1936), spawning salmon (Olivereau, 1954; Robertson and Wexler, 1960) and spawning ice-goby (Tamura and Honma, 1970). Moreover, in both the sexually mature and immature cod which do not need to regulate a heavy osmotic change in the Barents Sea, a continued high level of thyroid activity is maintained during the long migration (Woodhead, 1959a, 1959b). Nearly identical situation is detected in the landlocked Koayu living in Lake Biwa (Honma and Tamura, 1963). An extensive experiment was performed on the salinity preference of the stickleback, and marked seasonal differences were brought about, in addition to the salinity preference altered by use of both thyroid hormone and antithyroid drug (Baggerman, 1957).

The present examination revealed that the highest activity of the thyroid gland of the threespine stickleback occurs at the early time of ascending the river. The same results have already been reported in the cases of young Ayu (Honma, 1959 a) and spawning ice-goby (Tamura and Honma, 1970). Accordingly, the role of the thyroid hormone on the migratory behavior or active locomotion of the fish is suggested. However, it is difficult to decide whether hyper thyroid function is exclusively related to the increased demands of the hormone for the intense locomotion or to other sorts of factors, such as sexual maturation as speculated by Sage (1973) and external environment.

The reason that the thyroid of the juvenile stickleback underwent the mild state during the present transfer experiment might be elucidated by some more precise procedure. Histological pictures and salinity preference in relation to thyroid activity of the seaward migrants of the Pacific salmon are variable (Hoar and Bell, 1950; Baggerman, 1960, 1963). Nearly identical facts were secured by us about the thyroids of the seaward migrant of the chum salmon (*Oncorhynchus keta*) with mild picture, and of the yamame (*O. masou*) with active picture (Honma, unpublished data, see, Uchida, 1974). Therefore, it is probable that the thyroid hormone may be not so essential for the downstream migration of the Japanese marine type of threespine stickleback. In addition to these thyroidal changes, basophil cells supposed to be the thyrotrophs were not demonstrated light-microscopically in the hypophysis of the same specimens caught from the spawning bed and transferred into the sea water (Honma et al., 1976).

Much more studies throughout the year are needed to know the annual seasonal change of the thyroid gland of the landlocked form. However, it is difficult to explain the significance of well developed thyrotrophs in the hypophysis of the landlocked form (Honma et al., 1976), while the present examination revealed the mild thyroid activity during and after the breeding season.

A conspicuously tumorous thyroidal growth in the subpharyngeal region of the marine type of stickleback retained in the fresh water for a long time is of particular interest. The histologic pictures of the present case differ from those of Hamada (1975) who described the enlarged follicles containing a lot of colloid in the same type of stickleback kept in the fresh water for 110 days. However, the pictures illustrated in his paper seem to be a kind of the colloid goiter as an early developmental stage of adenomatous goiter (Meissner and Warren, 1969). A number of spontaneous adenomatous tumors of the thyroid gland were reported both in freshwater and marine teleosts (Gorbman and Gordon, 1951; Nigrelli, 1952; Mac Intyre, 1960; Lightner and Meineke, 1975), and their histologic pictures are well coincide with the present case of stickleback.

It is very difficult to mention the causal factor of the occurrence of the landlocked form. In our experiment, maintenance of the marine type of juvenile stickleback in the fresh water for a long duration after its normal downstream period brings about the thyroid adenoma associated with an exophthalmus and dwarfism of body. Low iodine content in lake water may contribute to hyperplastic thyroid tissue of coho salmon in Lake Michigan (Hoar, 1973; Drongowski et al., 1975). In the remora, *Echeneis naucrates*, reared for a considerable time in the sea water aquarium, Schlumberger and Lucké (1948) surmised that the development of the thyroid goiters may be responsible to some metabolic idiosyncrasy, since the goiters were never recognized in any other fishes kept under identical conditions. Therefore, the thyroid function of the fish that can live in two quite different environments are the difficult problem to be proved in near future.

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両側回遊に伴うイトヨの甲状腺の組織変化

本間 義治・塩田 清二・吉江 紀夫

イトヨの生活史の各段階における，甲状腺の組織像にみられる変化から，その役割を知る目的で，観察を進めた．甲状腺の活動は，海または塩水湖(加茂湖)中で機能低下状態にあり，遡河初期にもっとも高い．産卵場の親魚では濾費像がみられ，経産魚では退行変化が著しい．産卵場付近で得た稚魚は，比較的穏やかな状態を示すが，これら稚魚を海水槽に移すと，甲状腺は不活性化する．一方，会津盆地産陸封型イトヨを産卵期から秋までにわたって調べたところ，成魚・稚魚

共に穏やかな像が得られた．降海型イトヨの稚魚を，降海期以降も長く淡水槽中で飼育を続けると，顕著な甲状腺腫が発生した．すなわち，腺上皮の著しい肥厚と増殖および血管系の発達を伴い，コロイドは欠失状態を呈する．この腫瘍発生の原因について，考察を試みた．

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