Seasonal Changes in the Thymus of the Viviparous Surfperch, *Ditrema temmincki*, with Special Reference to Its Maturity and Gestation

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Abstract Thymic activity of the viviparous surfperch, *Ditrema temmincki*, was studied histologically throughout its life-span in relation to sexual maturity and gestation period. The materials used were caught on the coast of Aikawa Town, Sado Island in the Japan Sea, between June, 1978 and February, 1980. One hundred and sixty-six individuals were examined, including 123 adults, 16 juveniles and immatures and 27 fetuses. Males attain sexual maturity in August and begin mating. Mature eggs appear in November, and embryos, 1.5 mm in total length, are seen in the following January. Since parturition occurs in June, the gestation period is suspected to be 6 months. The peak enlargement of the thymus in both sexes of adult individuals occurs in June with another lower peak appearing in October. There is a distinct difference in the thymic activity between the adult male and female in the early gestation period. From January to February, the thymic volume of females is remarkably larger than that of males. During this time, the thymus of the fetus is difficult to see due to its undifferentiated condition. The differentiation of fetal thymus begins in March when the fetus reaches 10 mm in length. The mitotic figures of the large- and medium-sized lymphocytes are seen in this period. Toward April, small lymphocytes appear. The thymus of female fish is retained in an atrophic condition. However, a tremendous increase in its volume occurs in May and June, with a simultaneous increase in the thymic volume of the developing fetus. After parturition in June, the thymic volume of larval fish decreases month by month. In view of the relationship between the thymic volume of mother fish and fetus, the gestation period may be classified into three different periods: early (January to February), middle (March to April) and late (May to June).

Through our serial study of the thymuses of fish and cyclostomes, it is clear that thymic activity continues normally even in adult and aging fish (Tamura, 1978). A seasonal pattern with the peak thymic hyperplasia occurring in June was found in many fishes, such as 7 species of gobies (Tamura and Honma, 1973, 1974, 1975, 1977; Tamura et al., 1981), char *Salvelinus leucomaenis*, rainbow trout *Salmo gairdneri* and the medaka *Oryzias latipes* (Tamura, 1978). This coincidence in cyclic pattern of the thymic enlargement among these fishes invites some questions as to what are thymic functions. It is still unknown whether or not any relationship exists between thymic activity and sexual maturity, although it is well known that thymic involution occurs with sexual maturation.

Recently, it was discovered that in the viviparous cyprinodont (the guppy) the peak thymic enlargement appears in the fry soon after parturition. This event is followed with an involution of the thymus in males but a similar involution never takes place in females. It was also noticed that gravid fish with embryos in the eyed-period have a rather hyperplastic thymus (Tamura and Honma, 1979).

In order to investigate further the role of the thymus, we selected another viviparous fish, *Ditrema temmincki* Bleeker, for study. The period of sexual maturity in this species differs between the sexes, and the gestation period proceeds for 6 months from winter to early summer. The first objective of this study is to determine the periodicity of thymic activity both in the adult and fetus. The second is to find out if correlation of thymic activity exists between a gravid fish and its fetus.

Thus, histological changes in the thymus were examined from materials collected throughout the year. It is interesting to make comparison of thymic activities between oviparous and viviparous fishes.

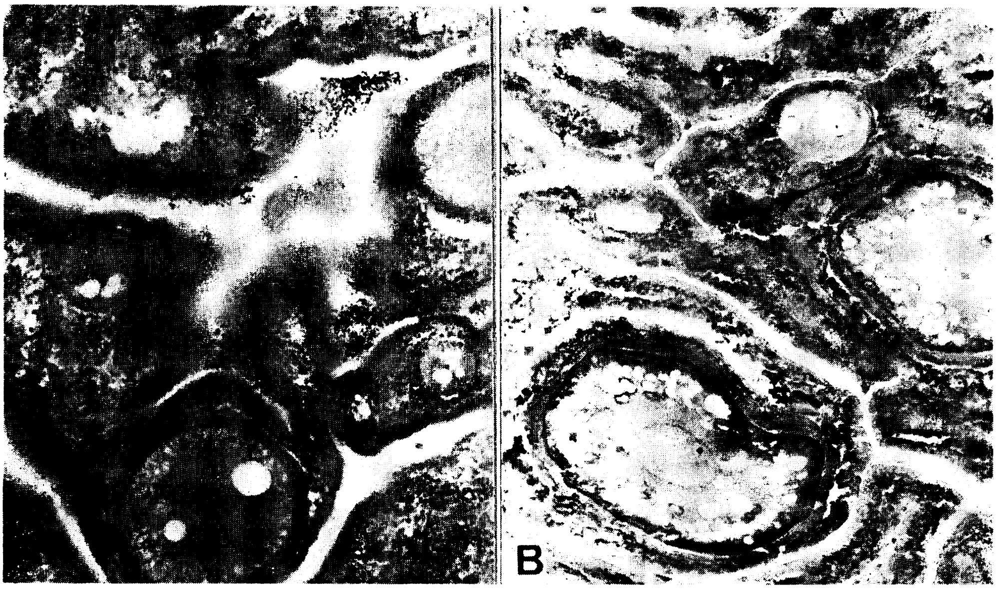


Fig. 1. A portion of the ovary of the adult surfperch, *Ditrema temmincki*. A: A female caught in August. Sperm masses are seen in the ovigerous folds. ×160. B: A female caught in November, showing eggs in the yolk vesicle stage with distinct zona radiata. ×120.

Material and methods

The surfperch, *Ditrema temmincki*, was collected mainly from the vicinity of the Sado Marine Biological Station of Niigata University located on the north-western coast of Sado Island in the Japan Sea. The collection was made at monthly intervals from June, 1978 to February, 1979. The materials used consisted of 123 adults, 16 immatures and juveniles and 27 fetuses.

Whole specimens were immersed in Bouin's solution. The block containing the thymus and gonads of adult fish, the thymus of immatures and juveniles, and the entire fetus were then dehydrated with alcohol, embedded in paraplast (Sherwood), cut serially at 8 μm thick, and stained with hematoxylin-eosin, azan trichrome and aldehyde fuchsin (AF). To estimate the rate of thymic activity, an area ($\times 10^4$) across the widest cross section of the thymic parenchyma was applied. This area was measured with a planimeter.

Results

Seasonal changes in the gonads. In the testis of surfperch caught at Sado Island, the spermatids begin to appear in July, and the sperm were conspicuous in August. Active courtship (chasing) was seen during skin diving from September to October. Sperm balls were seldom detected in the ovigerous folds of adult females caught in August (Fig. 1A). The ovum at this time was still in the yolk vesicle stage, but in the fall, some eggs began to show a distinct zona radiata and attained ca. 280～300 μm in diameter (Fig. 1B). After 6 months of gestation, parturition occured in late June. The growth rate of newborn fish (ca. 50～60 mm in length) was surprisingly high. They became sexually mature in December of the same year and measured 120～160 mm in length.

Changes in the thymus of adult fish. In the thymic parenchyma of gravid females (bearing fetuses measuring 10 mm in total length) caught in March and consisting chiefly of numerous lymphocytes and a few reticular cells, medium-size lymphocytes (4.2 μm in nucleus diameter) were dominant while small lymphocytes were difficult to see. Moreover, these small lymphocytes were difficult to recognize in the basal vessel system (Fig. 2A). The thickness of the parenchyma was 250～280 μm in this stage.

The histological make-up of specimens of both sexes caught in April was nearly the same as in females in March. In addition to an increase in the number of small lymphocytes, the presence of myoid cells was noticed in the parenchyma of female individuals caught in May. Although the thickness of the parenchyma had increased to 350～500 μm, the number of small lymphocytes (2.6 μm in nucleus diameter) was still scarce in the basal vessel system (Fig. 2B–1, 2). A nearly identical picture was encountered in the male.

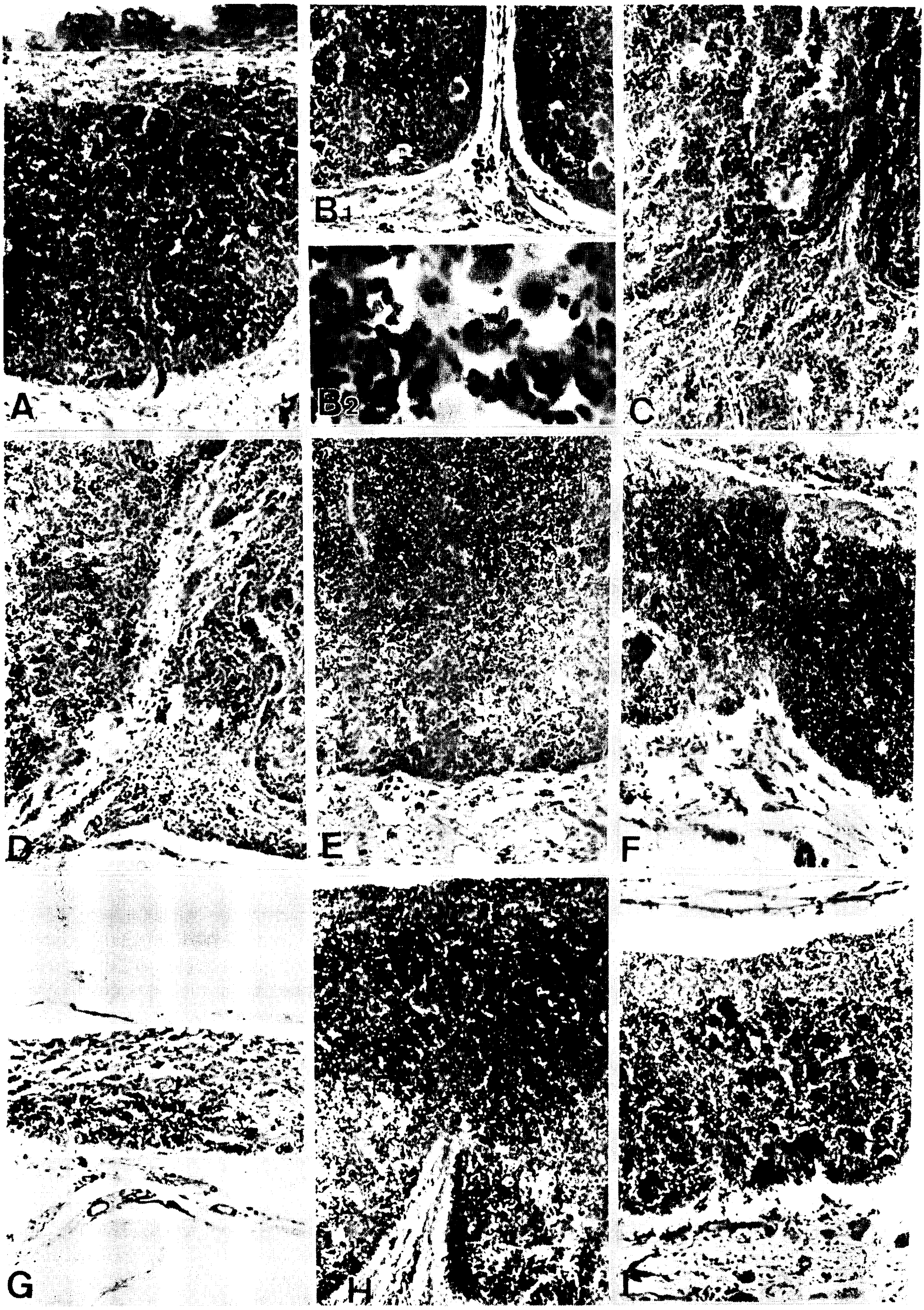
At the delivery period in June, a remarkable enlargement of the female thymus occurred; it measured 900 μm in thickness. The parenchyma was occupied exclusively with small lymphocytes at this time, numerous small lymphocytes had also entered the basal vessel system, indicating an active phase of lymphocyte liberation (Fig. 2C).

Subsequently, involution of the thymus was detected with a decrease in the number of small lymphocytes. A considerable number of small lymphocytes were still seen in the basal vessel system of the thymus in July. Discharge of small lymphocytes was hardly recognized in August specimens, and the area where these cells were scarce became wider (Fig. 2D, E). In September the whole parenchyma of both sexes showed a regressive change (Fig. 2F). This change became more remarkable month after month, and by December the parenchyma thickness was merely 85～119 μm (Fig. 2G). However, in January, a remarkable enlargement occurred in the parenchyma of a gravid female bearing an embryo 1.5 mm long. The parenchyma, 430 μm in thickness, was occupied by a large amount of small lymphocytes. These cells were also seen in the basal vessel system (Fig. 2H). On the other hand, the thickness of the parenchyma of male fish caught in January was only about half of that of the gravid female (Fig. 2I). This sexual difference in the thymus volume was even greater in February; the enlarged thymus in the gravid female with an embryo of 7 mm in total length was twice as large as the January specimens. No change was discerned in male fish (Fig. 3A, B). In other words, the histological make-up of January-February male was not much different from that of April.

Figure 4 indicates the changes in thickness of the thymus, as plotted on the area ($\times 10^4$)

Fig. 2. Cross section of the thymuses of the adult fish. A: A gravid female with fetuses (10 mm in total length) caught in March. Numerous medium-size lymphocytes exist in the parenchyma, while small lymphocytes are hardly visible in the basal vessel system. ×270. B-1: A gravid female caught in May. ×270. B-2: Enlarged view of the same thymus showing development of myoid cells in the parenchyma. ×800. C: A gravid female caught in June. Note existence of numerous small lymphocytes in the basal vessel system. ×270. D: A female caught in July after parturition. A considerable number of small lymphocytes still exist in the basal vessel system. ×270. E: A spent fish caught in August. Small lymphocytes are scarce in the basal vessel system. ×270. F: An adult fish caught in September. Note the diminishing parenchyma. ×270. G: An adult fish caught in December. Fertilization occurs within the ovary during this month. Note the remarkable involution of the parenchyma. ×270. H: A gravid female with fetuses of 1.5 mm in length, caught in January. Note the enlargement of the thymus and numerous small lymphocytes in the basal vessel system. ×270. I: A male caught in January. Note the difference in the thickness of the parenchyma between the female and male. ×270.

Fig. 3. Cross section of the thymuses of the adult, fetus and juvenile fish. A: A gravid female caught in February. Note further enlargement of the parenchyma. ×270. B: A male caught in February. The thickness and histological condition of the parenchyma are similar to those caught in January and March. Small lymphocytes in the basal vessel system are hardly detected. ×270. C: An embryo measuring 1.5 mm in total length, obtained in January. Eye vesicle and yolk mass are seen, but the thymic tissue is yet to be formed. ×170. D-1: A fetus measuring 10 mm in total length, obtained in March. Location of the thymus is as in the adult. ×170. D-2: Enlarged view of the same. Large lymphocytes are exclusively located in the parenchyma. ×680. E: A fetus measuring 37 mm in length, obtained in May. Note a marked increase in the number of small lymphocytes in the parenchyma. ×270. F: A fetus measuring 58 mm in length, obtained in June shortly before parturition. An increase in the small lymphocytes is more remarkable in this parenchyma. ×270. G: A juvenile caught in December. Note the diminished parenchyma. ×270.



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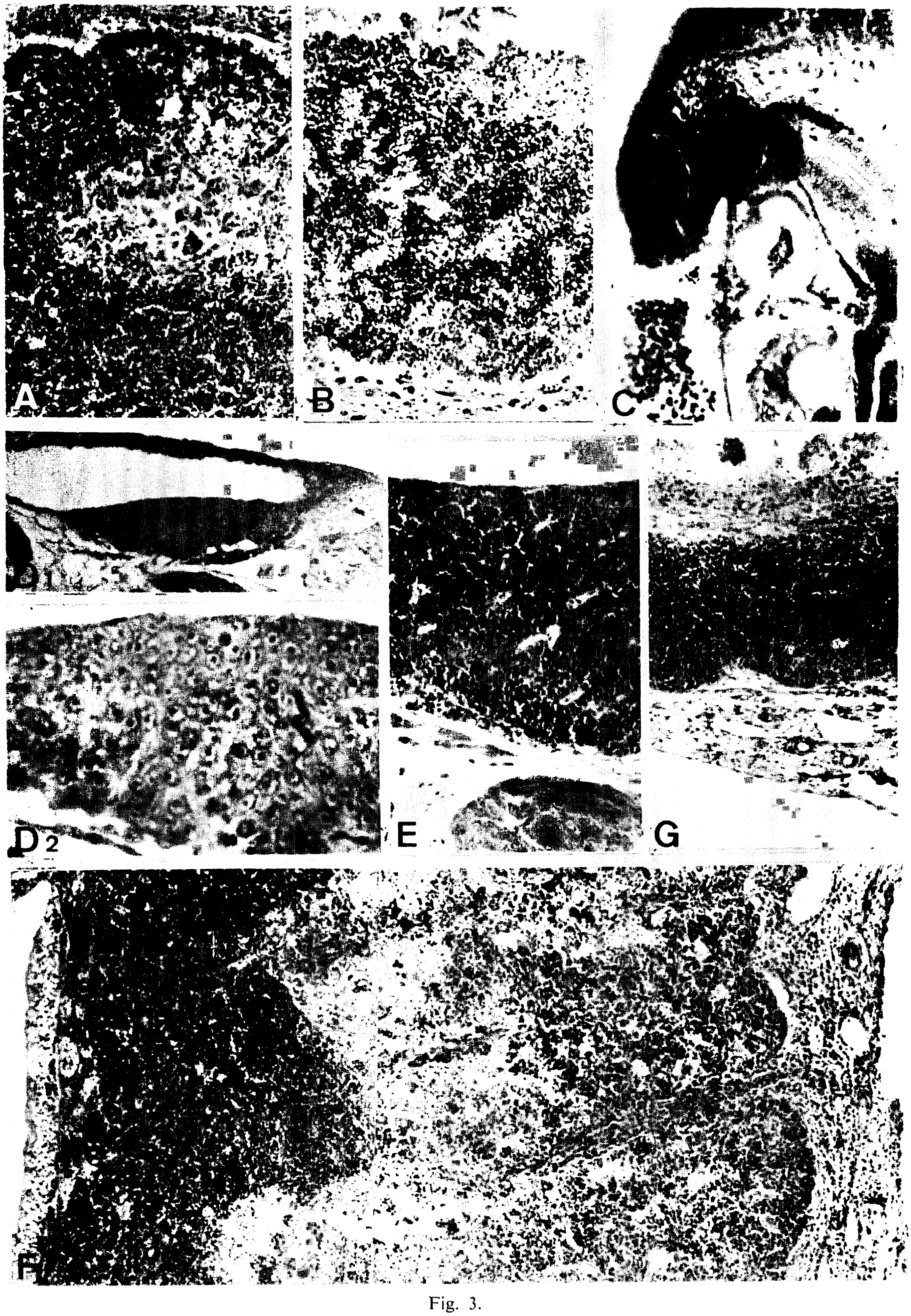

Fig. 3.

across the widest cross section. There was a clearcut seasonal change in both sexes with the maximum hyperplasia of thymus in June and the minimum (the most depressed thymus), in December. Subsequently, from January to February an increase in the thymus volume occurred with a distinct sexual difference. The time of a drastic increase in the female coincided with the commencement of gestation. In addition, a slight increase in thymus volume in both sexes was also noticed in October (Fig. 4A).

Changes in the thymus of underyearling fish including fetus, juvenile and young fish. In January the fetus measured merely 1.5 mm in total length (Fig. 3C). In February, with the formation of lenses it reached 7 mm in total length, however, no thymus tissue was recognizable. Toward March, the body length of the fetus became 10～12 mm, and the thymus was detectable in the same site as in adult fish. Mitotic figures of large lymphocytes (6.9 μm in nucleus diameter) were seen, but small lymphocytes had not made their appearance. The depth of the parenchymatous part measured 65 μm (Fig. 3D–1, 2). The body length of the fetus in April was about 22 mm, and mitotic figures of medium-size lymphocytes were occasionally seen in the parenchyma. The depth of the parenchyma (90～110 μm) was nearly twice as thick as specimens in March.

A remarkable enlargement of the thymic parenchyma (250～420 μm) was encountered in the fetus (37 mm in length) examined in May, and an increase in the number of small lymphocytes was also noted (Fig. 3E). Both increments in depth (450～750 μm) of thymus and number of small lymphocytes were greater in a fetus measuring 58 mm in length examined in June just before parturition. Meanwhile, liberation of small lymphocytes into the basal vessel system was marked, as well as the state of the mother fish in the same period (Fig. 3F). The area of the parenchyma with only a few small lymphocytes became wider and wider in the thymuses of juvenile fish caught in July and August. Subsequently, a gradual diminishing in the thymic parenchyma occurred, with the greatest diminution appearing in December, becoming only 200～400 μm in depth (Fig. 3G). This regressive change is similar to that of the adult fish.

Changes in the thymus of the underyearling shown in Fig. 4 indicates that the lowest level is in March and April when thymus tissue is recognized. Its subsequent enlargement occurring in May and June is in close parallel with the thymic enlargement of the mother fish.

Although a high level of thymus depth was maintained for a while (July and August), many areas had few small lymphocytes. Subsequent changes in the curve of underyearling is nearly identical with those of adult fish. It is well known that a rapid increase in weight and length occurs in juvenile fish. Accordingly, to indicate clearer thymic change, the ratio of cross area to body weight was calculated and plotted (Fig. 4Ba, Bb). According to this graph, the relative changes of thymus in the underyearling is much greater than in adults. A sharp decline in the thymus volume after parturition can also be noticed in this graph.

Discussion

It is interesting to note that in many species of fishes which we have examined throughout the years, a peak thymic activity always occurs in July in both sexes. This peak is independent of the breeding season in every species studied by Tamura and Honma (1973, 1974, 1975, 1977, 1979), Tamura (1978), and Tamura et al. (1981). The present investigation on the viviparous surfperch was suspected to yield the same result. As stated in the results, it is known that the male surfperch matures in August, the egg ripens in November or December, and the parturition is in June. Therefore, based on the above results, the seasonal pattern of the thymic activity in surfperch is also independent of the seasonal cycle of sexual maturity. Many investigators have reported the presence of a seasonal thymic activity in adult animals of higher vertebrates (Anderson, 1970; Galletti and Cavallari, 1972; Hightower, 1975; Ward and Kendall, 1975; Baak and Kater, 1975; Hussein et al., 1978a, b, 1979). However, the role and real siginificance of this cyclic change is still unclear.

A slight increase in the thymus depth found in surfperch caught in October was noticed. This tendency of brief interruption in thymic involution is also recognized in several species of fishes (Tamura and Honma, 1974, 1975, 1977;

Tamura. 1978).

It is particularly notable that the cyclic pattern of thymic activity in fish coincides well with the annual fluctuation of day length in Niigata district (Tamura and Honma, 1974, 1975, 1977; Tamura, 1978). A series of experiments on eyeless and pinealectomized medaka *Oryzias latipes* reared in a dark room during early summer also supported this finding. Rasquin and Rosenbloom (1954) demonstrated that a marked involution of the thymus and spleen occurred when a Mexican characin was maintained for a year or more in the dark. Nearly identical results were reported in the thymus of the koayu *Plecoglossus altivelis* reared under artificially controlled light (Honma and Tamura, 1972). However, it was somewhat difficult to explain the results, since in the case of koayu the thymic change was always accompanied with sexual maturity. On the other hand, a controversial result was obtained in the thymus of medaka maintained at high temperature. Even in the winter when the thymus was atrophied, a remarkable enlargement was produced when the medaka was at 25°C for a long (16 h/day) photoperiodic regimen (Tamura, 1978).

At present, it is still unclear whether or not a possible route may exist in the diencephalic hypothalamo-hypophyseal system in fish, although such a possibility was reported from mammals (Pierpaoli and Besendovsky, 1975; Deschaux et al., 1975), in addition to the route or relation to the pineal body (Csaba and Barath, 1975).

A distinct difference in the thymic activity between the male and female surfperch is of particular interest, since such a dissimilarity is unknown in oviparous fishes (Tamura and Honma, 1970, 1974, 1975, 1977; Honma and Tamura, 1972; Tamura, 1978; Tamura et al., in preparation). In a freshwater viviparous fish, the guppy, a rapid involution of the thymus occurs only in the male about one to two months after the highest enlargement. There is a correlation between the thymic enlargement and gestation, although in the guppy the gestation period is only 1 month. The thymic volume of a female during the later period of pregnancy with well-developed embryos is larger than that of females in other states (Tamura, 1978; Tamura and Honma, 1979). This finding suggests a possible interac-

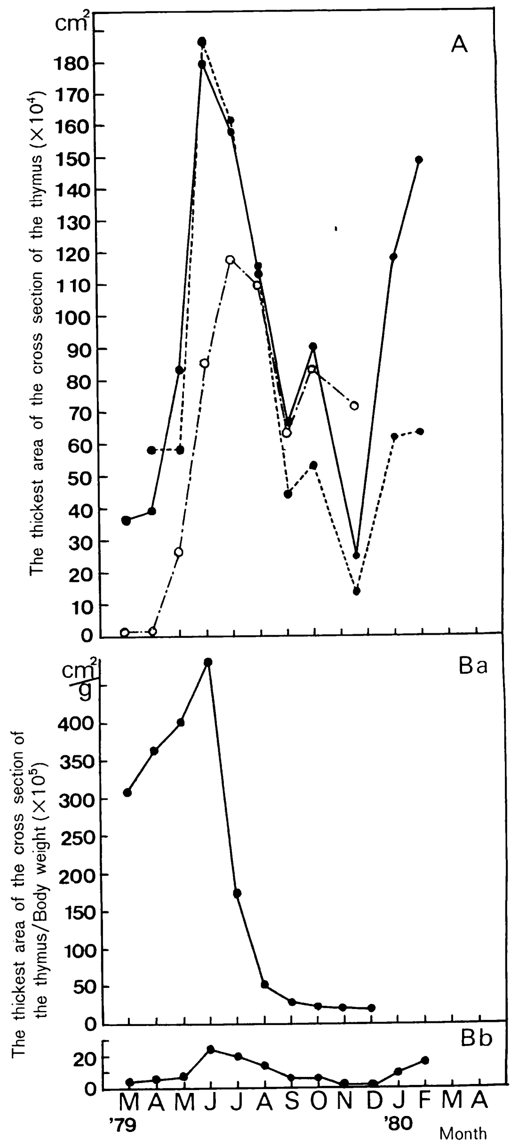

Fig. 4. Seasonal changes of the thymus depth. A: Graph plotted on the thickest area ($\times 10^4$) across the cross section. Solid line, female; dotted line, male; and broken dotted line, fetus and iuvenile. B: Graph plotted on the ratio of cross area to body weight ($cm^2/g \times 10^5$). a, fetus and juvenile; b, adult.

tion of thymic activities between the mother guppy and fetuses.

The undifferentiated thymus in the surfperch fetus was from January to February when the thymic enlargement of the mother fish was taking place. As described for the American surfperch by Engen (1968), the Japanese surfperch also has its thymic differentiation and lymphocyte multiplication occurred in March and April when the fetus reaches 10 mm. Thereupon, changes in the fetal thymus synchronize well with those of the mother fish.

During a considerably long period of gestation (6 months) mutual immune responses, as well as endocrine interactions, may have taken place between the mother and the fetal surfperch. In this connection, the gestation may be divided into three periods: the early (January～February), middle (March～April) and late (May～June), according to their thymus volume.

Engen (1968) demonstrated that histologically well-defined thyroid and pituitary occurred earlier than the thymus. Such an earlier differentiation of the hypothalamo-hypophysial neurosecretory system and late differentiation of the thymus was also observed (Tamura and Honma, unpublished data).

Sexual difference in thymic activity has already been described for rats of the Wister strain (Oda, 1964). The thymus growth rate of the female rat surpassed that of the male. However, the sexual difference in the thymic enlargement of the surfperch and guppy occurred only during their early phase of pregnancy. The reason why this phenomenon occurs only in viviparous fish is yet to be solved.

Literature cited

Anderson, W. L. 1970. Seasonal changes in thymus weights in ring-necked pheasants. Condor, 72(2): 205～208.

Baak, J. P. A. and L. Kater. 1975. The central thymus area. A hitherto undescribed region in the guinea-pig thymus. Koninkl. Nederl. Akad. Wetenschap. Amsterdam C, 78 (2): 123～129.

Csaba, G. and P. Barath. 1975. Morphological changes of thymus and the thyroid gland after postnatal extirpation of pineal body. Endocrinol. Exp., 9: 59～67.

Deschaux, P., G. Morel, P. Binimbi-Messengo and R. Fontanges. 1975. Study on the hypothalamo-hypophyso-thymic axis in the rat. Arch. Int. Physiol. Biochem., 83 (3): 423～434.

Engen, P. C. 1968. Organogenesis in the walleye surfperch, *Hyperprosopon argenteum* (Gibbons). Calif. Fish Game, 54 (3): 156～169.

Galletti, G. and A. Cavallari. 1972. The thymus of marmots: spontaneous, natural seasonal thymectomy? Acta Anat., 83: 593～605.

Hightower, J. A. 1975. DNA synthesis in the thymus of the adult newt, *Notophthalmus viridescens*. Acta Anat., 92: 454～466.

Honma, Y. and E. Tamura. 1972. Studies on the endocrine glands of a salmonoid fish, the Ayu, *Plecoglossus altivelis* Temminck et Schlegel–VIII. Degenerative changes in the thymus of Koayu exposed to artificial photoperiods. Bull. Japan. Soc. Sci. Fish., 38 (9): 995～1005.

Hussein, M. F., N. Badir, R. El Ridi and M. Akef. 1978 a. Differential effect of seasonal variation on lymphoid tissue of the lizard, *Chalcides ocellatus*. Develop. Comp. Immunol., 2: 297～310.

Hussein, M. F., N. Badir R. El Ridi and M. Akef. 1978 b. Effect of seasonal variation on lymphoid tissues of the lizards, *Mabuya quinquetaeniata* Lieht. and *Uromastyx aegyptia* Forsk. Develop. Comp. Immunol., 2: 469～478.

Hussein, M. F., N. Badir, R. El Ridi and M. Akef. 1979. Lymphoid tissues of the snake, *Spalerosophis diadema*, in the different seasons. Develop. Comp. Immunol., 3: 77～88.

Oda, M. 1964. Age changes in the blood-forming tissues in the albino rat of a subline of the Wister strain. Arch. histol. Japon., 25 (2): 145～163.

Pierpaoli, W. and H. O. Besendovsky. 1975. Role of the thymus in programing of neuroendocrine functions. Clin. Exp. Immunol., 20 (2): 323～338.

Rasquin, P. and L. Rosenbloom. 1954. Endocrine imbalance and tissue hyperplasia in teleosts maintained in darkness. Bull. Amer. Mus. Nat. Hist., 104 (4): 359～426, plates 4～23.

Tamura, E. 1978. Studies on the morphology of the thymus in some Japanese fishes. Spec. Publ. Sado Mar. Biol. Stat., Niigata Univ., (1): 1～75. (In Japanese with English summary).

Tamura, E. and Y. Honma. 1970. Histological changes in the organs and tissues of the gobiid fishes throughout the life-span—III. Hemopoietic organs in the ice-goby, *Leucopsarion petersi* Hilgendorf. Bull. Japan. Soc. Sci. Fish., 36 (7): 661～669.

Tamura, E. and Y. Honma. 1973. Histological changes in the organs and tissues of gobiid fishes throughout its life-span—V. Seasonal changes in the branchial organs of the flat-head goby in relation to sexual maturity. Bull. Japan. Soc. Sci. Fish., 39 (10): 1003～1011.

Tamura, E. and Y. Honma. 1974. Histological changes in the organs and tissues of the gobiid fishes throughout their life-span—VI. Seasonal changes in the lymphopoietic organs of the flat-head goby. Bull. Japan. Soc. Sci. Fish., 40 (5):

447～455.

Tamura, E. and Y. Honma. 1975. Histological changes in the organs and tissues of the gobiid fishes throughout their life-span—VII. Seasonal changes in the hemopoietic organs of the fork-tongue goby. Bull. Japan. Soc. Sci. Fish., 41 (4): 413～422.

Tamura, E. and Y. Honma. 1977. Histological changes in the organs and tissues of the gobiid fishes throughout their life-span—VIII. Seasonal changes in the thymus of four species of gobies. Bull. Japan. Soc. Sci. Fish., 43(8): 963～974.

Tamura, E. and Y. Honma. 1979. Seasonal changes in the thymus of young guppy. Abstract, Ann. Meet. Japan. Soc. Sci. Fish., April, 1979, 101. (In Japanese).

Tamura, E., M. Maruyama and Y. Honma. (In preparation) Histological changes in the organs and tissues of the gobiid fishes throughout their life-span—IX. Seasonal changes in the thymus of *Inu koma* in relation to sexual maturity.

Ward, P. and M. D. Kendall. 1975. Morphological changes in the thymus of young and adult red-billed queleas, *Quelea quelea* (Aves). Phil. Trans. Roy. Soc. London, B, 273: 55～64.

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成熟ならびに妊娠との関係からみたウミタナゴ胸腺の周年変化

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佐渡産ウミタナゴの胎仔，幼魚，成魚多数を材料として，胸腺活動を成熟ならびに妊娠期間との関係を考慮しながら組織学的に観察した．雄は8月に成熟し，交尾を始めるが，雌では成熟卵が11月に出現し，翌年1月に 1.5 mm の胚が発育し，6月に産出する．雌雄とも親魚の胸腺は6月に最も肥厚し，その後回復するが，10月には再び少し肥厚する．妊娠初期の1～2月には，雌親魚の胸腺が一時著しく肥厚し，明瞭な性差を示すが，胎仔のそれは未分化状態にある．胎仔胸腺は3月頃(体長 10 mm)から分化し始め，4月に完成する．一方，この頃の雌親魚の胸腺は再び萎縮していたが，出産期には親魚・胎仔共に急速に肥厚する．しかし，産出後の仔魚胸腺は退縮を始める．このような母体と胎仔の胸腺肥厚の関係からみると，妊娠期間の胸腺活動は3期に区分され，その理由は今後の解析をまたねばならない．

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