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Comparative Studies on the Maturation Process of Two Types of *Cottus nozawae*—I. The Annual Cycle of Ovarian Development

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Abstract Monthly changes in the ovaries of the small-egg type (SE) and the large-egg type (LE) of *Cottus nozawae* were compared biometorically and histologically. The relative weight of ovaries (GSI) scarcely differed between two types for six months after spawning in May to November. The GSI values were slightly higher in the SE than in the LE during the period of December to January. In the spawning period (April), however, the relation was inverse, because of the rapid increase in the ovary weight of the LE since February. The diameter of ovarian eggs in the spawning period was significantly larger in LE than in SE. From a comparison of the monthly changes of egg sizes between the two types, it is definite that the difference was a result mainly of the rapid increase of egg sizes in the LE after March. Vitellogenesis in the LE became active in early August, and in mid September for SE. The beginning of formation of yolk globules also differed between LE and SE.

Thus, there are two types of *C. nozawae*, each with unique modes of ovarian development. The mode in the LE is not simply an extention of development in the SE.

Cottus nozawae is a freshwater cottid fish, first reported by Snyder (1911) from the Ishikari River in Hokkaido. Recently, Goto (1975a, b) carried out detailed investigations on the life history and distribution of *C. nozawae* and divided this species into two types based on ecological and morphological evidence. They were named, for convenience, as the small-egg type (SE) and the large-egg type (LE) respectively. The former type, SE, inhabits the lower reaches of a river and spawns a large number of small eggs from which swimming larvae hatch. In contrast, LE lives in the middle and upper reaches of the river and spawns a small number of large eggs from which well-developed benthic larvae hatch. It was suggested that the LE was adaptively derived from some groups of the small-egg type, with a large increase in the size of the mature eggs. A similar phenomenon, to that found in the two types of *C. nozawae*, has also been reported in some freshwater fishes and shrimps, such as *Cottus pollux* (Mizuno and Niwa, 1961), *Rhinogobius brunneus* (Mizuno, 1960) and several species of *Atidae* and *Palaemonidae* (Shokita, 1975). In all cases, the studies suggested that increased egg size might be necessary for the land-locked species to be able to spend their whole life in a mountain stream or spring. Thus, it is important to clarify annual changes in the growth of ovarian eggs in both types of *C. nozawae*, to understand the mechanism of increased egg size and the problems of parallel speciation.

Histological studies of the annual cycle in the development of ovaries in fish have been done in the minnow (Matthews, 1938), flounder (Yamamoto, 1956a), whiting and the Norway pout (Gokhole, 1957), goldfish (Beach, 1959; Yamamoto and Yamazaki, 1961), ayu-fish (Honma and Tamura, 1961), and rainbow trout (Yamamoto et al. 1965).

In this study, the relative weight of ovaries, the size of ovarian eggs and histological changes of the ovaries were compared monthly between the two types of *C. nozawae*, in order to understand how and why the size difference in the mature eggs in both types evolved.

Material and Methods

Field collections of *Cottus nozawae* were made monthly from August, 1976 to July,

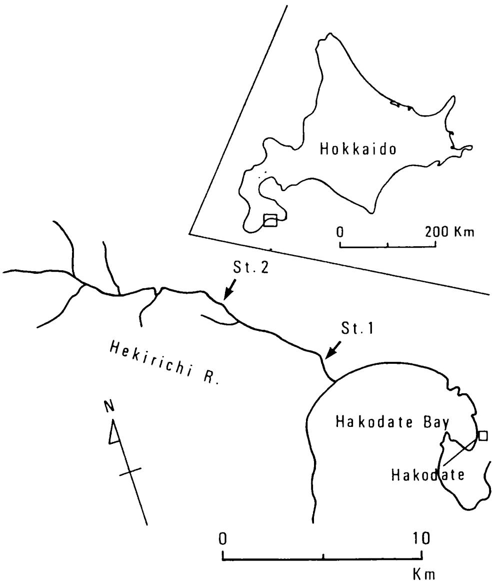


Fig. 1. Location and map of Hekirichi River. St. 1: station at which the small-egg type were captured, St. 2: station at which the large-egg type were taken.

1977 in the Hekirichi River, which flows through the southernmost part of Hokkaido and drains into Hakodate Bay. SE fish were captured with a dip net in the lower reaches of the river, whereas LE were taken from the upper reaches (Fig. 1).

Female adults, older than 2 years of age were selected from total catches, and were measured for body length, body weight and gonad weight for calculation of the gonad-somato index (B.W./B.L.^{3} $\times 1000$). In addition, the diameter of eggs taken in November to April collections was measured in twenty eggs for each individual.

The gonads of three to five females were fixed monthly with Bouin's fluid for histological observations. Serial tissue-mat 8 μ sections of the gonads were cut, and stained with Delafield's hematoxylin and eosin.

Results

Monthly changes in gonad weight. The monthly changes in gonad weight are shown in Table 1 and Fig. 2 as gonad-somato index

Table 1. Collection date, number of specimens, body length and gonad-somato index of adult females of two types of *C. nozawae* examined in the present study.

Type	Date	Number of specimens	BL (mm)	GSI(Mean)	
Small-egg type (SE)	Aug. 5	7	108.2～82.2	0.181～0.319	(0·233)
	Sep. 16	9	144.7～99.7	0.197～0.428	(0.298)
	Oct. 16	12	106.6～91.6	0.350～0.538	(0.433)
	Nov. 19	4	117.3～93.7	0.783～1.244	(1.059)
	Dec. 29	7	118.1～95.8	1.720～2.340	(1.994)
	Jan. 30	4	106.7～95.1	1.935～3.217	(2.636)
	Feb. 27	7	122.2～88.0	2.242～3.904	(2.930)
	Mar. 29	8	125.8～90.2	4.091～6.081	(4.984)
	Apr. 14	6	127.2～80.0	2.232～5.922	(4.059)
	May 20	3	128.6～85.5	0.368～0.802	(0.531)
	June 20	3	109.1～86.4	0.391～0.494	(0.440)
	July 14	5	114.7～86.4	0.157～0.322	(0.243)
Large-egg type (SE)	Aug. 5	7	89.5～75.2	0.261～0.436	(0.344)
	Sep. 16	8	93.8～70.0	0.321～0.455	(0.410)
	Oct. 16	7	94.2～81.1	0.481～0.709	(0.572)
	Nov. 19	13	92.9～77.9	0.596～1.100	(0.717)
	Dec. 29	4	88.1～78.7	0.919～1.868	(1.167)
	Jan. 30	7	88.8～70.9	0.933～2.369	(1.421)
	Feb. 27	6	98.9～77.5	1.706～3.713	(2.408)
	Mar. 29	4	85.0～76.2	2.955～5.957	(4.411)
	Apr. 14	9	85.3～75.6	4.260～7.734	(5.959)
	May 20	3	83.0～69.6	0.611～1.474	(1.024)
	June 20	4	83.5～78.1	0.363～0.900	(0.523)
	July 14	5	90.9～79.6	0.431～0.862	(0.556)

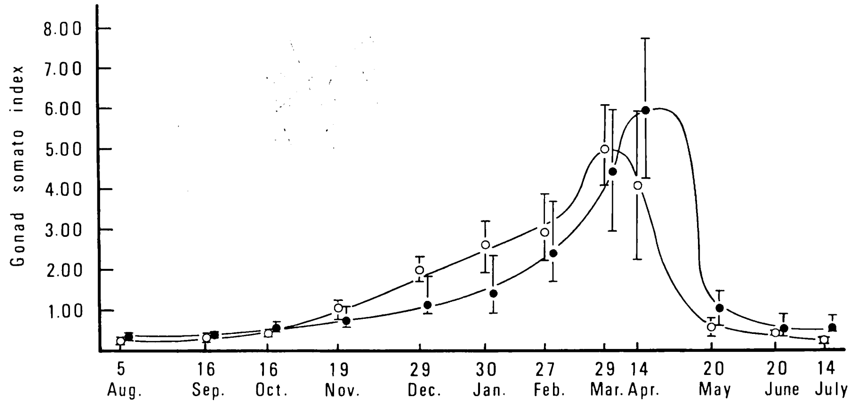


Fig. 2. Seasonal changes of gonad-somato index in female adults of two types of *C. nozawae*. ○ small-egg type, ● large-egg type. Vertical bars indicate range of variation.

(GSI) in two types of *C. nozawae*. In SE, the values of GSI were lower than 0.5 from June to October, and the lowest value was shown in early August (0.23). The value increased slightly to 1.06 in November. The gonad weight increased markedly during the period of December to March. The values of GSI was 1.99 in late December, 2.64 in late January and 2.93 in late February, and reached to the maximum by late March (4.98). The average GSI in mid April (4.06), just before the spawning, was slightly lower than that in late March, but the difference was not significant ($P > 0.05$). After spawning, the gonad weight decreased suddenly and the value of GSI in late May was 0.53.

The monthly changes of gonad weight in LE had a pattern similar to that of SE. That is, the values of GSI in June to October were less than 0.6, and the lowest value was found in early August (0.34). The gonad weight gradually increased from the beginning of November and reached a maximum in mid April (5.96). In mid May just after spawning, the gonad weight decreased suddenly (1.02).

In spite of the similarity above, however, there were some important differences in the changes of gonad weight in LE when it is compared to SE. The increasing rate of GSI for the first three months was lower in LE than in SE (Fig. 2), though the gonad weight began to increase in both types in November. The values of GSI of the former were significantly lower than those of the latter in December and January ($P < 0.05$). Moreover, the time when the maximum GSI occurred was different in the two types, namely by mid April in LE and by late March in SE. The mean GSI value at the maximum of the former was slightly higher than that of the latter.

Monthly changes in the size of ovarian eggs. The size of ovarian eggs in both types (Fig. 3) increased clearly in diameter each month up to the spawning periods. The increasing rate, however, was different between types, and was higher in LE. From late November to late February the size of eggs in LE was only slightly larger than that of SE, as can be seen by their ranges, which partly overlapped each other; but, in March the ranges separated clearly. In mid April,

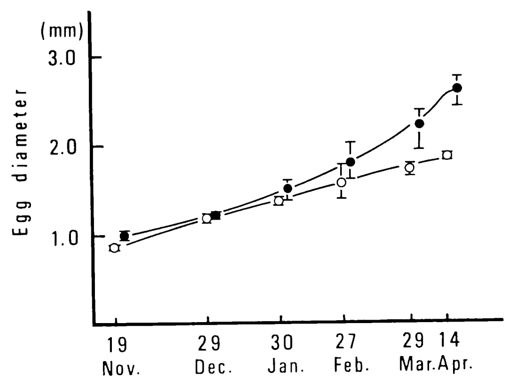


Fig. 3. Monthly changes of egg diameter in two types of *C. nozawae*. ○ small-egg type, ● large-egg type. Vertical bars indicate range of variation.

just before the spawning period, the mean egg diameter in LE was 2.60 mm compared with 1.83 mm in SE.

Histological changes. The annual cycle in development of oocytes in both the small-egg type and the large-egg type are shown schematically in Fig. 4.

In August, the ovaries of SE were composed of a few oogonia (6～10 μ) and many oocytes at the chromatin nucleolus stage (15～18 μ) and the peri-nucleolus stage (180～240 μ) (Fig. 5A). This is about 3.5 months after spawning. The ovaries contained some oocytes at the yolk vesicle stage (320～390 μ) in mid September, and some large oocytes at the same stage (410～500 μ) in mid October (Fig. 5B, C). The oocytes in the primary yolk stage were 650 μ in size and appeared in November. From November to December, yolk globules accumulated rapidly (Fig. 5D), and in late January many oocytes (1050～1150 μ) in the tertiary yolk stage were in the ovaries (Fig. 5E). In addition to the oocytes, the ovaries also contained a large number of oocytes at younger stages, such as the primary and secondary yolk stages, yolk vesicle stage, peri-nucleolus stage and chromatin nucleolus stage. In February, many oocytes in the migratory nucleus stage were found in the ovaries (Fig. 5F). The oocytes in this stage were 1300～1500 μ in diameter and many yolk globules were fused together to make large yolk masses. In late March, the prespawning period, the ovarian eggs were nearly ripe (Fig. 5G). The yolk masses continued to fuse together and formed a few large yolk masses in the ovarian eggs. The size of eggs in the ovaries, however, hardly differed from that in the previous month, and the ovaries contained still many oocytes in the yolk vesicle stage, peri-nucleolus stage and chromatin nucleolus stage. In mid May shortly after spawning, the ovaries were composed of atretic eggs and the residual oocytes in the peri-nucleolus, chromatin nucleolus or

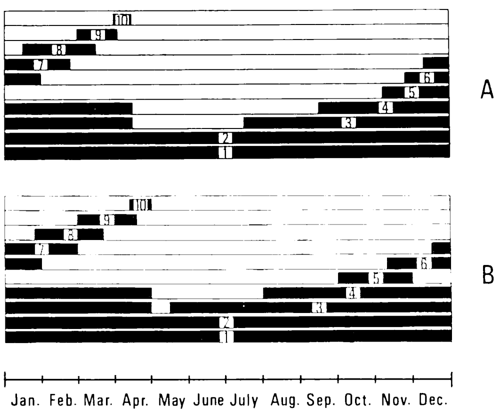
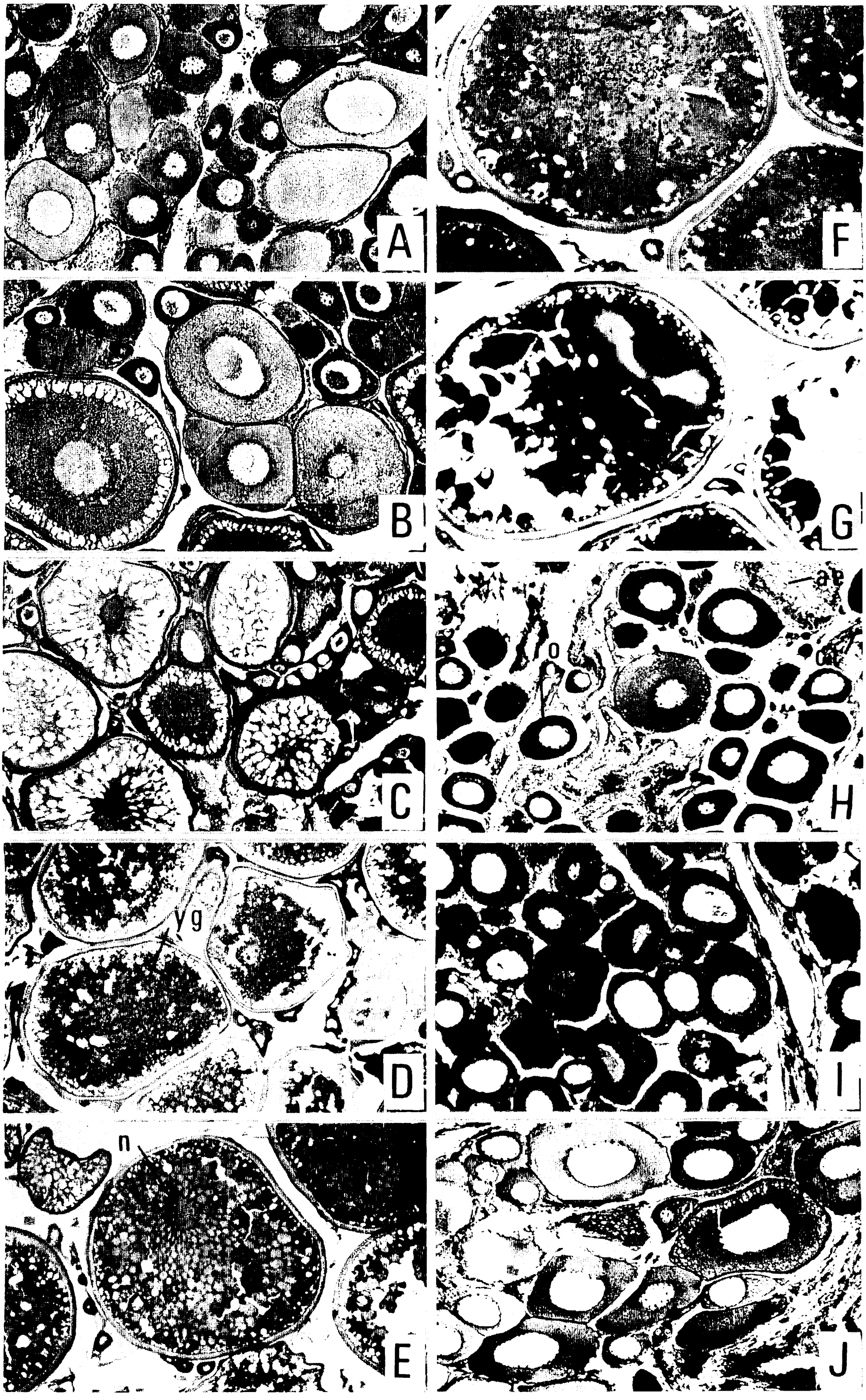


Fig. 4. Schematic diagrams showing the annual cycle in development of oocytes of two types of *C. nozawae*.
A: small-egg type, B: large-egg type. 1. Chromatin nucleolus stage; 2. Early peri-nucleolus stage; 3. Late peri-nucleolus stage; 4. Yolk vesicle stage; 5. Primary yolk stage; 6. Secondary yolk stage; 7. Tertiary yolk stage; 8. Migratory nucleus stage; 9. Pre-maturation stage; 10. Ripe egg stage.

Fig. 5. Photomicrographs of the ovaries in the small-egg type. All figures are cross sections.
A: An ovary in early August, showing oocytes at the early and late peri-nucleolus stage. ×93. B: An ovary in mid September, showing oocytes at the yolk vesicle stage and peri-nucleolus stage. yv, yolk vesicle. ×93. C: An ovary in mid October, showing oocytes at advanced phase of the yolk vesicle stage and other young stages. ×56. D: An ovary in late December, showing oocytes at the secondary yolk stage and other young stages. yg, yolk globule. ×37. E: An ovary in late January, showing oocytes at the migratory nucleus stage, the tertiary yolk stage and other young stages. n, nucleus. ×37. F: An ovary in late February, showing oocytes at the migratory nucleus stage and early peri-nucleolus stage. ×37. G: An ovary in late March, showing eggs at the pre-maturation stage. ×37. H: An ovary in mid May, showing atretic eggs, the residual oocytes and the connective tissue. ×93. I: An ovary in mid June, showing the residual oocytes and the connective tissue. ×93. J: An ovary in mid July, showing a similar condition to the previous figure. ×93.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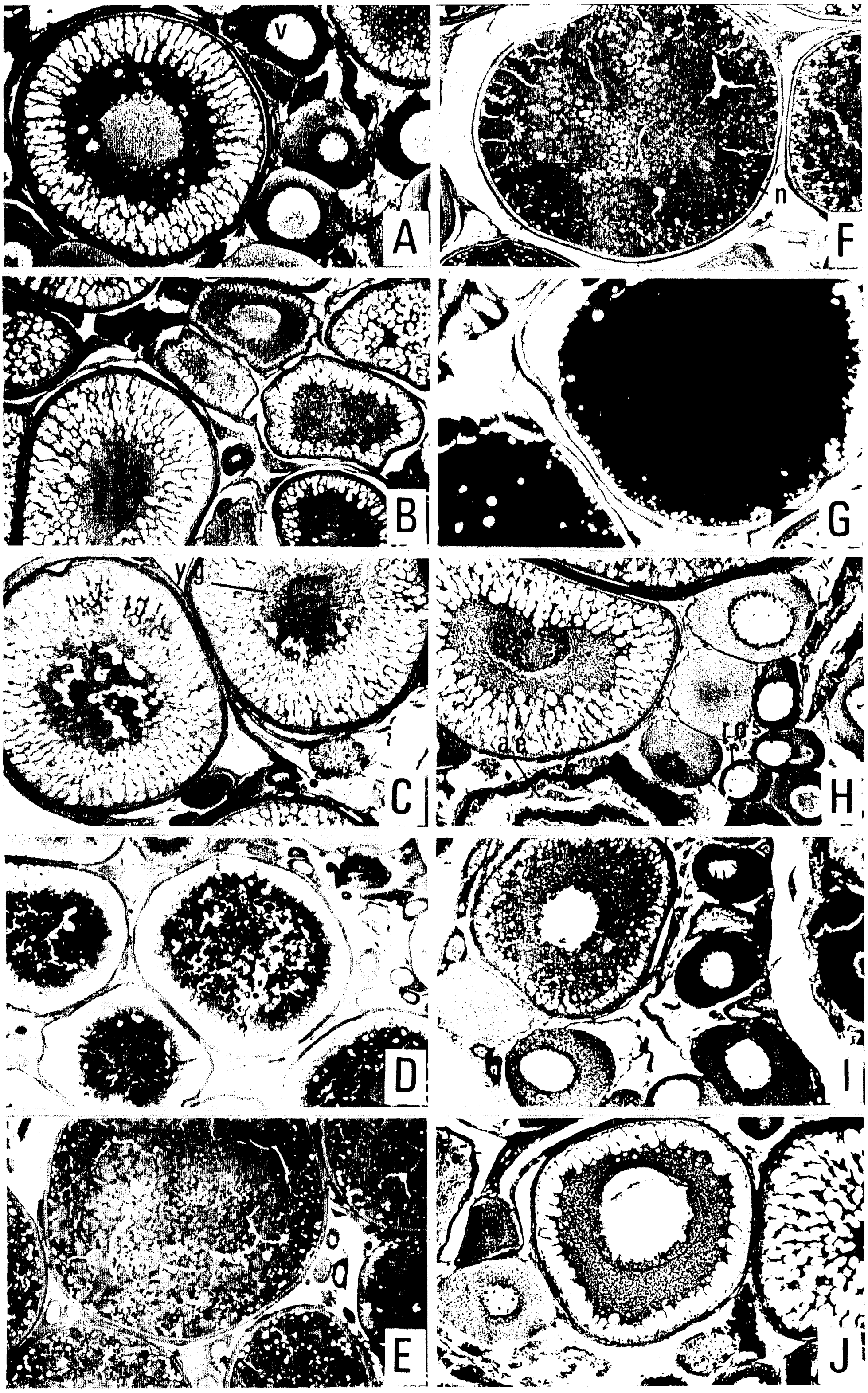
yolk vesicle stages (Fig. 5H). The atretic eggs and the oocytes at the yolk vesicle stage were gradually absorbed and finally disappeared (Fig. 5I, J). The ovaries were filled with a few oogonia and many oocytes at the peri-nucleolus stage and chromatin nucleolus stage in June and July.

In contrast, the maturation process of LE was remarkably different from that of SE. In this type, the ovaries in early August, about 3 months after spawning, already contained some oocytes at the yolk vesicle stage, with many oocytes at the peri-nucleolus and chromatin nucleolus stages (Fig. 6A). Therefore, vitellogenesis in LE began about one month earlier than in SE. The size of oocytes at the yolk vesicle stage in this month was $420\sim530\mu$ in diameter. The ovarian eggs grew further (Fig. 6B), and in mid October some of them were about 800μ in diameter and began to contain yolk globules (Fig. 6C). From November to December, yolk globules rapidly accumulated (Fig. 6D), and in late January many ovarian eggs arrived at the tertiary yolk stage or the migratory nucleus stage (Fig. 6E). The well-developed eggs of the LE in January were almost at the same stage as those of the SE. The size of eggs in the LE, however, were $1200\sim1370\mu$ in diameter, and were slightly larger than in the SE ($1050\sim1150\mu$). In LE, the ovarian eggs continued to increase in size (Fig. 6F), and were about 2000μ in diameter in late March (Fig. 6G) and the yolk globules fused together and formed some large yolk masses. In mid May shortly after the spawning, the ovaries contained atretic eggs and the residual oocytes, similar to the small-egg type in the same month (Fig. 6H). Then, the atretic eggs were gradually absorbed and disappeared (Fig. 6I, J). Though the oocytes in the yolk vesicle stage remained in the ovaries after spawning, they were clearly degenerating. Thus, the ovaries in June and July were fundamentally composed of oocytes at the peri-nucleolus and chromatin nucleolus stages, and oogonia.

Discussion

This study showed that the development of ovaries of both the large- and the small-egg types of *Cottus nozawae* could be classified as partial synchronism. This has already been demonstrated in many species of fish with multiplicity of spawning in a lifetime, such as the herring (Hickling and Rutenberg, 1936; Yamamo, 1956b), speckled trout (Valdykov, 1956) and flounder (Yamamoto, 1956a). It was found, however, that there were differences in time in the appearance of active vitellogenesis and in the beginning of yolk accumulation between two types of *C. nozawae*. Judging from the presence of oocytes at the yolk vesicle stage, vitellogenesis in the LE appears to become active in early August, while in the SE it appears in mid-September. Moreover, in the LE the forma-

Fig. 6. Photomicrographs of the ovaries in the large-egg type. All figures are of cross sections.
A: An ovary in early August, showing a few oocytes at the yolk vesicle stage and many oocytes at the peri-nucleolus stage. yv, yolk vesicle. ×93. B: An ovary in mid September, showing oocytes at advanced phase of the yolk vesicle stage and other young stages. ×56. C: An ovary in mid October, showing oocytes at the primary yolk stage and other young stages. yg, yolk globule. ×56. D: An ovary in late December, showing oocytes at the secondary yolk stage and other young stages. ×37. E: An ovary in late January, showing oocytes at the tertiary yolk stage and other young stages. ×37. F: An ovary in late February, showing oocytes at the migratory nucleus stage and tertiary yolk stage. n, nucleus. ×37. G: An ovary in late March, showing eggs at the pre-maturation stage. ×37. H: An ovary in mid May, showing an atretic egg and the residual oocytes. ae, atretic egg; ro, residual oocyte. ×93. I: An ovary in mid June, showing the atretic eggs, the residual oocytes at the yolk vesicle stage and oocytes at the peri-nucleolus stage. ×93. J: An ovary in mid July, showing a similar condition as the previous figure. ×93.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tion of yolk globules in oocytes started in October, while in the SE it started in November. This means that the yolk formation begin earlier in the LE than in the SE. The GSI is not significantly different between the two types during the period of May to November. The GSI, however, from December to January was significantly lower in LE than in SE, suggesting that the growth rate of the ovaries of the two types are different during the winter season. This difference in growth rate of the ovaries at this season can reflect ovarian egg development and as a result the oocytes in the ovaries of the SE are in the same or a more developed stage as compared with those of the LE in late February. After that, the ovarian eggs of the SE do not show an increase in the quantity of yolk to arrive at the ripe stage, while those of the LE continue to increase in quantity of their yolk until they reach the final stage of development. This fact is further supported by the results obtained from the measurement of egg diameter. Namely the egg sizes in the two types separate clearly from each other in March. It is reasonable to presume that the differences of the mature egg size between the two types result from the difference in the amount of yolk deposition which occurred about two months before spawning.

These differences in ovarian development suggest that the two types of *C. nozawae* have already had type specific modes in the development of ovaries or ovarian eggs. Up to date, there have been few studies on differences of the egg size in animals. Thorson (1950) and Marshal (1953) suggested, through their investigations in marine invertebrates and fishes, that in closely related species the egg size might be related to the water temperature in their environments. In general, species living in lower water temperatures had larger eggs. On the other hand, Lack (1954) pointed out that birds tend to possess large-size eggs under the conditions of poor nutrition. In all cases, however, the relationship between egg size and nutrition or water temperature for different species has not been resolved.

As reported already (Goto, 1975b), the habitat of the LE is in the upper reaches of a river, while the small-egg type inhabits the lower reaches. The environmental conditions of the LE coincide with the conditions mentioned on marine invertebrates, fishes or birds with larger eggs; the upper reaches are generally of low water temperature and poor nutrition in comparison with that of the lower reaches (Mizuno, 1963; Mizuno and Gose, 1972). Goto (1977) pointed out that the LE developed quickly and dwarfed the SE. Therefore, it is presumed that the developmental mode of ovaries and ovarian eggs in the LE were formed at a branch of the evolutionary line of the SE. This occurred in the LE by connecting the change of the mode of growth and development in its ontogeny as an adaptation to the environment of the upper reaches of the river, and not by direct influence of environmental conditions to ovaries and ovarian eggs.

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ハナカジカの 2 型の成熟過程についての比較研究—I. 卵巣の発達の年周期

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卵の大形化の機構を解明する一環として，ハナカジカの小卵型と大卵型の卵巣の周年変化を比較した．比卵巣重量は，産卵後数カ月間は両型間にほとんど差がみられないが，12～1月にかけて小卵型は大卵型より若干大きくなる．しかし，2月以降大卵型の卵巣は急速に生長し，産卵期(4月)にはその関係は逆転する．産卵期における卵巣卵の卵径は，小卵型に比べ大卵型が明瞭に大きいが，それは主に3月以降の大卵型の卵巣卵の大きさの著しい増大によってもたらされる．

組織学的観察から，両型の卵巣の発達にはいくつかの重要な違いが存在することが明らかになった．つまり，大卵型では卵黄形式が8月初旬には既にアクティブになるのに対して，小卵型ではそれより1カ月以上後の9月中旬にアクティブになる．このような卵黄形成開始の時期のずれに伴なって，卵黄球形成の開始時期にも両型間でずれがみられた．

また2月以降産卵期まで，小卵型の卵巣卵は卵径があまり変化することなく卵黄球が融合して成熟期に達するが，大卵型では卵黄球の融合とともに卵径が一層増大し成熟期に達する．

以上の結果から，両型は既に固有の卵巣及び卵巣卵の発達様式を有しており，大卵型の発達様式は単に小卵型のそれを延長したものではないと結論された。

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