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Reproductive Cycle in Female Fusilier, *Caesio diagramma*

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농어목 어류, *Caesio diagramma* 암컷의 생식주기

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This study was conducted to study the reproductive cycle in female fusilier, *Caesio diagramma* by using the histological methods. Histological changes of the ovary were well correlated with the patterns of gonadosomatic index (GSI) and hepatosomatic index (HSI). GSI was increased in April as the value of HSI increase and reached to its maximum in May and June. Oocytes at the chromatin-nucleolus and peri-nucleolus stages were observed in the ovary throughout the year. In April, oocytes containing yolk appeared in ovaries of a few fishes. Most oocytes appearing in May and June belonged to the tertiary yolk stage. Frequency of oocytes appearance at the tertiary yolk stage in May and June was higher than that of the other months. Moreover, the empty follicles and atretic oocytes were observed in the ovaries with many vitellogenic oocytes during these two months. Thereafter, oocytes of the yolk stage disappeared in September. The spawning period of *C. diagramma* is from the month of April to June, and this species belongs to an asynchronous and multiple spawner.

Key words : Female fusiler, *Caesio diagramma*, Coral reef fish, Reproductive cycle, Asynchronous spawner

Introduction

Many studies on the reproductive cycles have been conducted in relation with the basic biology and aquaculture in teleost fishes. Histological studies have been made to clarify the developmental rhythm in ovarian eggs during reproductive cycle in teleosts (Yamamoto, 1958 ; Yamamoto and Yamazaki, 1961 ; Yamamoto et al., 1974 ; Yoon, 1981 ; Takemura et al., 1987).

Patterns of oocyte development in teleosts have been categorized into three groups ; the type of synchronous, the type of group synchronous, and the type of asynchronous spawners (Yamamoto and Yamazaki, 1961). These groups have been found mainly in the species inhabiting the temperate or subarctic zones. However, there are few studies on the dynamics of reproductive process of teleosts living in coral reef areas. This may be due to the diversity of fish species with low population density and relatively little economical importance. The vitellogenesis of the female fish is the most important phenomenon closely related to the growth and

maturity of oocyte.

Fusilier, *C. diagramma* is commercially important in Okinawa as one of the major reef fish species. Only one study has been done in this species with relation to fishery resources in the Ryukyu Islands (Kyan and Yamamoto, 1983). Comparative study on fusiliers *C. diagramma* is inhabiting in tropic or subtropic zones between the relevancy of ovary growth and quantitative change of vitellogenin during spawning period. This study has been designed to identify the characteristics of the annual reproductive cycle of the fish based on histological observations of ovaries.

Materials and Methods

Experimental fish

Samples of female fusilier *C. diagramma* used in this study were collected by fishing once a month from April to December, 1991, along the coast of Sesoko Island, Okinawa, Japan. They were kept overnight in running sea water in outdoor 45 ton tank at Tropical Biosphere Research Center, University of the Ryukyus until histological observations. After fishes were anesthetized in 0.01% ethyl p-aminobenzoate solution, body weight (BW), gonad weight (GW), and liver weight (LW) of each fish were measured by blotted dry weights. Gonadosomatic index (GSI, GW/BW×100) and hepatosomatic index (HSI, LW/BW×100) were calculated.

Histological procedures

Tissues of the ovary and liver were fixed in Bouin's solution. Following serial dehydrations with ethanol, tissues were embedded in paraffin. Serial sections of 7 μm were selected and stained with Delafield's hematoxylin-eosin solution for light microscopic observation. The periodic acid-Schiff (PAS) reaction was used for the detection of polysaccharides in each tissue in according to the method of McManus (1946). Oogenesis of *C. diagramma* was divided into the six successive stages according to the classification of Yamamoto (1956) ; chromatin-nucleolus, peri-nucleolus, oil-droplet, primary yolk, secondary yolk and tertiary yolk.

Results

Monthly changes of water temperatures

From April 1991 to March 1992, the water temperature changes of the sampling areas were recorded at the surface and 30 m depth (Fig. 1). The average temperature at the surface was 22.7℃ in April, increased steadily during the following months, reached to its peak at 30.4℃ in July. The temperature remained high until the month of September, after that it was rapidly decreased.

The mean temperature of water at 30 m depth was very similar to that of the surface.

Monthly changes of GSI and HSI

Monthly changes of GSI and HSI of *C. diagramma* are shown in Fig. 2. GSI was maintained relatively high from April (1.26±0.25%) to June (1.30±0.22%).

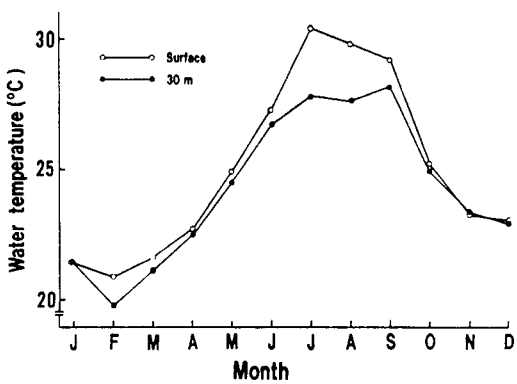

Fig. 1. Monthly changes of water temperature where samples were collected.

A rapid decrease of the value was observed in July (0.49±0.06%), maintained at bottom levels until the month of September for HSI and until December for GSI, respectively. From April to June, the changes in HSI almost coincided with fluctuation of GSI. The HSI values remained low from July to September and then gradually increased from the

following months till winter.

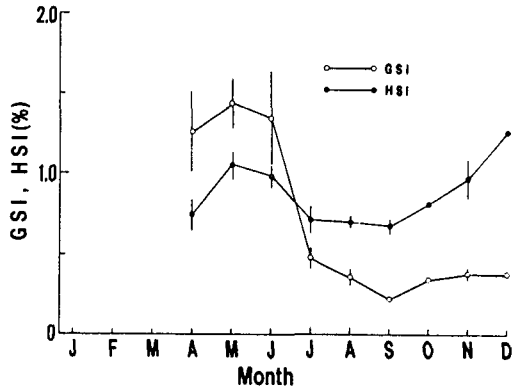

Fig. 2. Monthly changes of GSI and HSI in female fusilier *Caesio diagramma*. The each of mark with vertical bars indicates mean±SEM.

Morphological observation of oocyte development

(1) Chromatin-nucleolus stage (Fig. 3a)

The smallest oocytes of about 10 μm in diameter were found in just beneath the surface of ovigerous lamellae throughout the year. They had a very thin sheath in cytoplasm. In the nucleus a large nucleolus was occupied in the most part of the oocyte.

(2) Peri-nucleolus stage (Fig. 3b)

The size of the oocytes of peri-nucleolus stage was ranged from 20 to 110 μm in diameter. During the oocyte development, the cytoplasm increased gradually in volume, and showed a deeply staining with hematoxylin. Within the nucleus, there were many chromatin-threads and nucleoli around the inner margin of the nuclear membrane. Toward the end of this stage, the cytoplasm was decreased in affinity to hematoxylin.

(3) Oil-droplet stage (Fig. 3c)

The size of oocytes was ranged from 130 to 260 μm in diameter. Oil-droplets were appeared and increased rapidly in number and volume. The follicle layers of oocytes during this stage were thicker than those of the previous stage, in which the vitelline envelope also became evident.

(4) Primary yolk stage (Fig. 3e)

The oocytes at this stage were ranged from 110 to 220 μm in diameter. Oil-droplets were varied in size, and showed a formation of circular zone around the nucleus. Yolk globules appeared as small granules in the peripheral region of cytoplasm. The size of yolk globules was less than 1 μm in diameter and increased gradually both in number and size. Upon PAS staining, the yolk vesicles appeared slowly in the peripheral cytoplasm (Fig. 3d). The vitelline envelope (3 μm in diameter) was observed clearly as a membrane located between the cytoplasm and the follicle layer.

(5) Secondary yolk stage (Fig. 3f)

At this stage, the oocytes diameter was ranged from 200 to 330 μm. Oil-droplets of various size were located in the inner part of the cytoplasm. Yolk vesicles gradually moved outerwards and located at the periphery of the oocyte. The yolk globules increased in size and occupied two-thirds of the outer membrane of cytoplasm. Nucleoli of the nucleus were appeared to be distributed at random. The vitelline envelope and follicle layer in this stage became thicker than those in the primary yolk stage.

(6) Tertiary yolk stage (Fig. 3g)

As yolk globules gradually increased in number and size, the oocytes became larger ranging from 300 to 440 μm in diameter. The entire cytoplasm was filled with many yolk globules and oil-droplets which scattered in cytoplasm. The vitelline envelope was clearly observed and had almost same thickness (about 10 μm) as that in the previous stage (Fig. 3f). A small micropyle was observed sometimes in the thin vitelline envelope (Fig. 3j).

Monthly changes of oocyte composition

Developmental stages of oocytes are showm in Table 1 and Fig. 4. Oocytes at the chromatin-nucleus stage were excluded from counting, because they were countress.

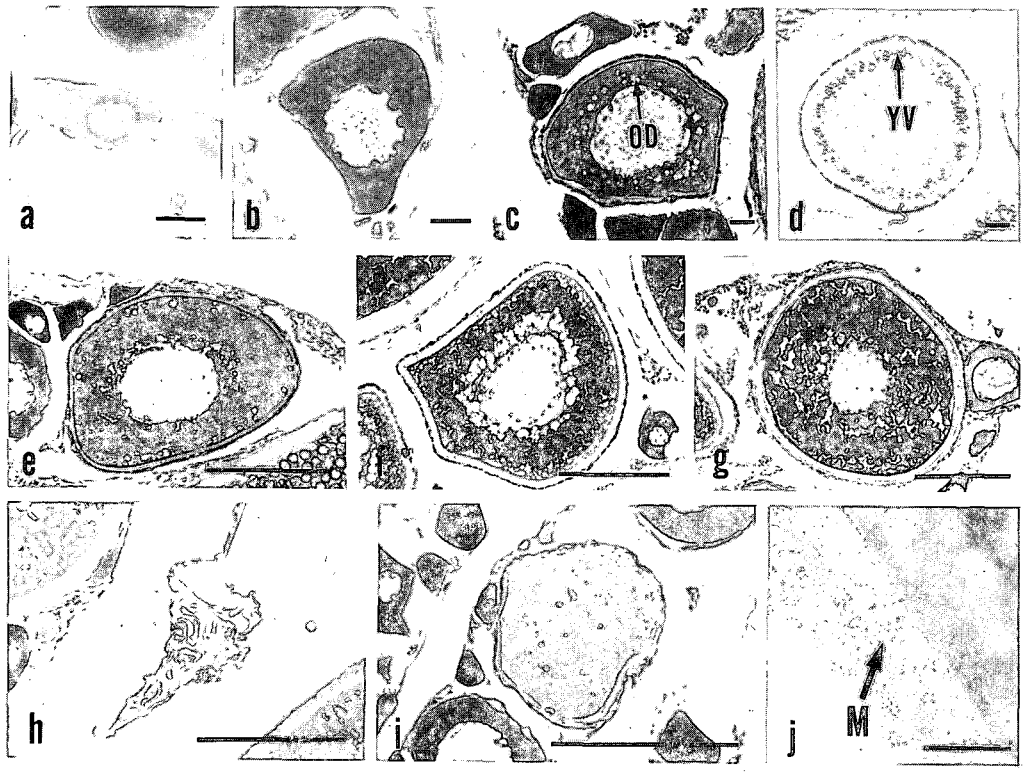

Fig. 3. Cross sections of each developmental stage in oocyts. a ; Chromatin-nucleolus stage, b ; Peri-nucleolus stage, c ; Oil-droplet stage, d ; PAS positive rection in oocyte, e ; Primary yolk stage, f ; Secondary yolk stage, g ; Tertiary yolk stage, h ; Empty follicle in an ovary, i ; Atretic oocyte in an ovary, j ; Micropyle of an oocyte in the tertiary yolk stage. Abbreviation, OD ; Oil-droplet. YV ; Yolk vesicle. M ; Micropyle. a~d ; Bars indicate 10 μm. e~j ; Bars indicate 100 μm.

Oocytes of the peri-nucleolus stage were observed throughout the year. In April, oocytes containing yolk were already observed in two out of three fish sampled (Fig. 5A & a). The most advanced oocytes were appeared at the tertiary yolk stage, whereas percentage of oocytes in this stage was quite low (0.3~0.4%). The other fishes sampled in April had oocytes only in the peri-nucleolus and the oil-droplet stage. In May and June, the oocytes of yolk stage were turned into tertiary yolk stage, reaching their perce ntage from 6.1 to 9.6%. On the other hand, oocytes in the peri-nucleolus stage were decreased in percentage (75.7~78.2%) (Fig. 5B & b). During these 2 months, the highest probation of empty follicles was found in the fish ovaries in May (Fig. 3h). Atretic oocytes were also detected in the ovaries from three fish in May and June (Fig. 3i). They had characteristics such as deformed shapes, yolk globules, and the radiata broken into fragments. In July, the percentage of peri-nucleolus stage of oocytes was 86.1% for all fish sampled. Although oocytes in the tertiary yolk stage were relatively low (3.1%), compared to those in June, oocytes containing yolk were still observed in the ovaries. All oocytes of the secondary and the tertiary yolk stage were disappeared from the ovaries. In August, oocytes in the primary yolk stage were low (0.2%) in the ovaries. Most of ovaries sampled from September to December contained oocytes at

Table 1. Monthly changes of number and percentage size of oocyte in each developmental stage of ovary

Month	No. of oocytes	Developmental stage of ovary										Empty* follicle	Atretic* oocyte
		Peri-nucleous stage		Oil-droplet stage		Yolk stage							
						Primary		Secondary		Tertiary			
		No.	%	No.	%	No.	%	No.	%	No.	%		
	948	934	98.5	14	1.5								
Apr.	2572	2389	92.9	92	3.6	47	1.8	33	1.3	11	0.4		
	3173	2874	90.6	130	4.1	98	3.1	62	1.9	9	0.3	+	
	6693	6197	94.1	236	3.1	145	1.5	95	1.1	20	0.2		
May	3819	2891	75.7	154	4.1	235	6.1	171	4.5	368	9.6	+++	++
	4917	4153	84.5	191	3.9	228	4.6	54	1.1	291	5.9	+	++
Jun.	4142	3134	75.7	372	9.1	242	5.8	118	2.8	276	6.6	+++	++
	3678	2737	74.4	479	13.1	136	3.7	108	2.9	218	5.9	++	+
	12737	10024	78.2	1042	8.7	606	4.7	280	2.3	785	6.1		
	3661	3633	99.2	23	0.6	5	0.1						
Jul.	3578	3503	98.1	60	1.6	13	0.4					+	
	3053	3003	98.4	39	1.3	11	0.4						
	10292	10141	98.5	122	1.2	29	0.3						
	2175	2163	99.5	5	0.2	7	0.3						
Aug.	2847	2842	99.8	3	0.1	2	0.1						
	2903	2898	99.8	1	0.03	4	0.2						
	8806	7903	99.7	9	0.1	13	0.2						
	1820	1819	99.9	1	0.1								
Sep.	2755	2753	99.9	2	0.1								
	1647	1647	100										
	6222	6219	99.9	3	0.1								
Oct.	1950	1950	100										
	2398	2398	100										
	4348	4348	100										
	2631	2631	100										
Nov.	2348	2348	100										
	1804	1804	100										
	6783	6783	100										
Dec.	2416	2416	100										

* Relative amount of empty follicle and atretic oocyte are indicated by marks, + to +++. The value after the underline in each column represents total number of each month.

the peri-nucleolus stage (Fig. 5C & c).

Discussion

Characteristic features of oogenesis in *C. diagramma* are in the formation of oil-droplets which is the first structure appearing prior to yolk vesicle formation.

Vitellogenesis have been described in several fishes such as *Lesbistes reticulatus* (Takano, 1964), *Lateolabrax japonicus* (Hayashi, 1972) and *Anguilla japonica* (Yamamoto

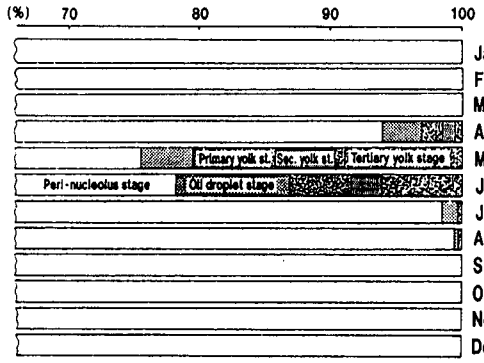

Fig. 4. Monthly composition of ovary in the fusilier, *Caesio diagramma* at developmental stage.

et al., 1974). It is known that yolk vesicles give rise to cortical alveoli at the later stages of oogenesis and they are involved with formation of the peri-vitelline space at fertilization (Laale, 1980). The paucity of yolk vesicles was noted for some viviparous fish, *L. reticulatus* (Takano, 1964), *Sebastes taczanowskii* (Takemura et al., 1987). In oviparous forms, on the contrary, the peri-vitelline space may be either wide or narrow depending on whether the eggs are pelagic or demersal (Laale, 1980). Probably, the paucity of yolk vesicles in *C. diagramma* may be related to the pelagic nature of the spawned eggs.

In the present study, oocytes at the migratory nucleus and more matured stages were not observed in any fish sampled during the reproductive period. The nucleus, however, is migrated toward the micropyle at the animal pole of the oocyte in these stages of *Carassius auratus* (Yamamoto and Yamazaki, 1961), *Rhodeus ocellatus* (Shirai, 1962), *Oryzias latipes* (Yamamoto and Yoshioka, 1964), *Theragra chalcogramma* (Yoon, 1981), *S. taczanowskii* (Takemura et al., 1987). It was reported that in *S. taczanowskii* the migratory nucleus stage continued more than two month. Conversely, the development of oocytes from the tertiary yolk stage to the ripe stage through the process of nuclear migration took place in about 12 hours in *O. latipes* (Yamamoto and Yoshioka, 1964). It is likely that the duration of the migratory nucleus stage was different among species according to their maturation rhythms. It seems to indicate that the pre-maturation and maturation stages may be of short duration after the commencement of nuclear migration as in the case of *O. latipes*.

The oocyte development of *C. diagramma* coincided well with changes of GSI. In April, various types of oocytes containing yolk were appeared in the ovaries. In the ovaries sampled in May and June, the mostly oocytes reached to the tertiary yolk stage and their percentage was relatively high. During these two months, GSI was sustained high. These results indicated that active vitellogenesis in *C. diagramma* continued during May and June. Kagawa and Takano (1979) observed characteristic features of ovulating follicles in *O. latipes* histologically, histochemically and cytologically, and proposed that the empty follicles existing in ovaries after ovulation could be used as an indicator of spawning. In the present study, many empty follicles and active vitellogenic oocytes were observed in the ovaries sampled in May and June. These features also suggested that active spawning occurred during these two months.

In the patterns of oocyte development in teleosts, the type of synchronous spawner is defined as fish in which all oocytes, once formed are spawned once in single event which is followed by death. *Oncorhynchus masou* (Yamamoto et al., 1959) have so far been ascertained to be this type.

The type of group synchronous spawner is defined as fish in which two groups of oocytes are recognized in an ovary near the spawning period ; a fairy synchronous clutch of developing oocytes and immature oocytes. Fishes of this type usually do spawning once a year and thus several times in a lifetime. *Liopsetta obscura* (Yamamoto, 1956) and *T. chalcogramma* (Yoon, 1981) are species belonged to this type.

The type of asynchronous spawners are fish containing various kinds of developing

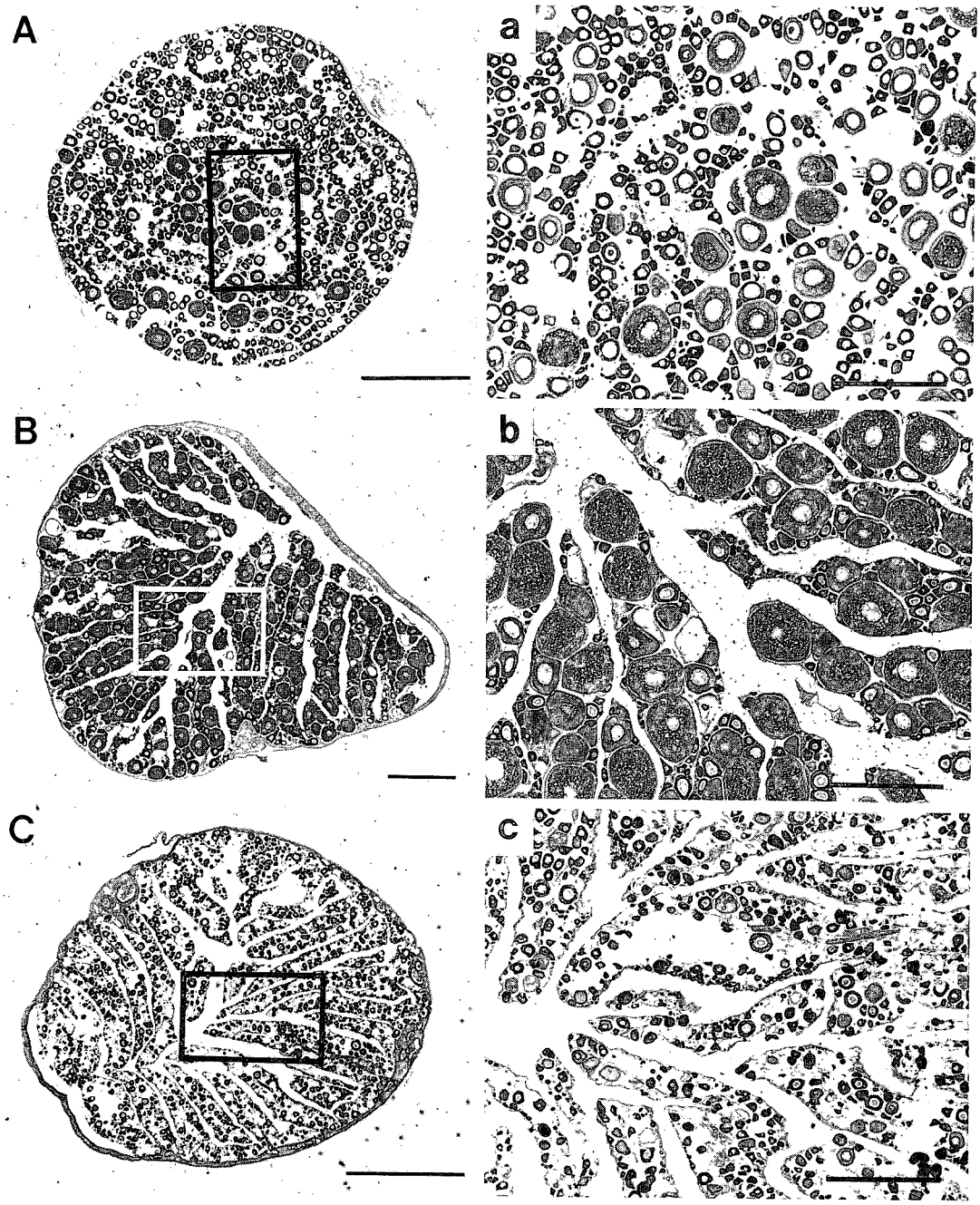

Fig. 5. Cross sections of ovaries in fusilier, *Caesio diagramma*. A ; Section of an ovary in April, a ; High power view of Fig. 5A (Pre-spawning season). B ; Section of an ovary in June, b ; High power view of Fig. 5B (Spawning season). C ; Section of an ovary in September, c ; High power view of Fig. 5C (After spawning season). A~C ; Bars indicate 1 mm, a~c ; Bars indicate 500 μm.

oocytes in ovaries and having a comparatively long spawning period, and ordinary spawning several times within a period. *Sardinops melanosticta* (Ishida et al., 1959), *C. auratus* (Yamamoto and Yamazaki, 1961) and *Blennius pholis* (de Vlaming, 1983) are reported to be belonged to this type. As mentioned above, in *C. diagramma,* active vitellogenesis was occurred simultaneously in the oocytes during developmental stages. Therefore, it is suggested that *C. diagramma* may belong to the type of asynchronous spawner.

In the present study, it was not clarified how many times *C. diagramma* spawned during its one reproductive season. The existence of several types of empty follicles may indicate the ovary during the active spawning. The frequency of spawning in this fish may be assessed by detailed histological and cytological observations of the empty follicles.

요 약

농어목 어류인 *Caesio diagramma*의 생식주기를 밝히기 위하여 난모세포의 발달과정을 조직학적 방법으로 관찰하였다. 난모세포의 발달과정은 염색인기, 주변인기, 유구기, 제1차 난황구기, 제2차 난황구기 및 제3차 난황구기의 6단계로 구분되었다. 염색인기와 주변인기의 난모세포는 연중 관찰되었으며, 난모세포 발달과정에서의 특징은 난소 내에서 유구가 초기에 형성되며, PAS 염색 결과 난황포는 타 어류에 비하여 크기가 작았고 양도 적었다. 4월에 채집된 어류의 난소에서는 난황 형성기의 난모세포가 출현하기 시작하였다. 5~6월의 난소에서는 제3차 난황구기 난모세포가 관찰되었으며, 연중 가장 높은 비율을 나타냈다. 또한, 이 시기부터 난소의 일부에서는 배란을 마친 여포와 퇴화중인 난모세포도 다수 관찰되었으며, 7월까지 이어졌다. 9월 이후의 난소에서는 난황 형성기의 난모세포가 관찰되지 않았으며, 12월까지 염색인기 및 주변인기의 난모세포가 주류를 이루었다. 생식소중량지수(GSI)와 간중량지수(HSI)는 4월부터 증가하기 시작하여, 5~6월에 최고치에 도달하였으며 GSI와 HSI의 변화는 난모세포의 발달과정과 일치하였다. 따라서 *C. diagramma*의 주산란기는 5~6월이며, 난소의 발달과정과 여러 형태의 여포가 존재하는 점으로 미루어, 본 종은 비동기발달형의 난소를 가지며 다회산란 어류에 속한다.

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