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Histological Observations of the Female Reproductive Cycle of Honeycomb Grouper, *Epinephelus merra* in Chuuk

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Chuuk에 서식하는 Honeycomb Grouper, *Epinephelus merra* 암컷의 생식주기

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ABSTRACT : The seasonal reproductive cycle of the female honeycomb grouper, *Epinephelus merra*, inhabiting Chuuk was examined by histological observations of the ovaries. The gonadosomatic index (GSI) began to increase in February and peaked in March. Histological observations revealed many oocytes laden with yolk in the ovaries from March to April. From June to January, the ovaries were occupied by immature oocytes. These results suggest that the reproductive season of *E. merra* in Chuuk is from March through April.

Key words : *Epinephelus merra*, Reproductive cycle, Grouper, Chuuk.

요 약 : 2000년 2월부터 2001년 2월까지 남태평양 미크로네시아 군도의 Chuuk 연안에 서식하는 *Epinephelus merra* 암컷을 대상으로 생식주기를 조사하였다. 생식소숙도지수 (GSI)는 2월부터 증가하기 시작하여 3월에 최고치 (3.41±0.84)를 보였다. 조직학적 관찰결과 3월과 4월의 난소 안에는 난황을 가진 다양한 단계의 난모세포와 배란여포들이 존재하였다. 6월부터 1월에 난소 안에는 미성숙 난모세포만 있었다. 이들 결과로 *E. merra*의 산란 시기는 3월과 4월로 유추할 수 있었다.

INTRODUCTION

The reproductive cycles of teleosts are species-specific reproductive strategies adapted to their environments. Spawning times can be divided into 1) spring, 2) spring and summer, 3) summer, 4) fall, and 5) winter (Aida, 1991). Groupers are protogynous hermaphroditic fish that inhabit coral reefs and reef zones in the tropics and subtropics; they belong to the family Epinephelinae, which includes 159 marine species in 15 genera (FAO, 1993). Groupers are highly regarded both as a food and as ornamental fish in Korea, China, Japan, and Southeast Asia (Tanaka et al., 1990; Shapiro et al., 1993; Ferreira. 1995). There are several ongoing studies examining grouper aquaculture and their sexual characteristics. To induce artificial sex reversal and obtain high-quality spermatozoa and fertilization, steroid hormones (*e.g.*, 17-methyltestosterone and 11-ketotestosterone) have been given to some groupers orally, by injection, or by implantation (Kuo et al., 1998; Tan-Fermin et al., 1994; Lee et al., 1996; Hwang et al., 1998). In addition to hormone treatments, social control has also been used to manipulate the expressed sex and sex ratios in *E. coioides*. Moreover, the relationship between spawning rhythm and the lunar cycle has been studied in some groupers (Quinitio et al., 1997; Lee et al., 2002; Michael, 2000).

E. merra is a small grouper that inhabits the coral reefs of Southeast Asia and the Pacific coast. This study used histological observations of the ovaries to assess the seasonal reproductive cycle of *E. merra* near the Chuuk coast of Micronesia in the South Pacific.

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MATERIALS AND METHODS

1. Sample Fish

E. merra were collected monthly using a hook and line off the coast of Chuuk Island, in Micronesia, in the South Pacific from February 2000 through February 2001. After first anaesthetizing the fish in 0.01% ethyl p-amino benzoate solution, body weight (BW) and gonad weight (GW) were recorded, and the gonadosomatic index (GSI: GW×100/BW) was calculated. In all, 233 fish were collected (total length 11.64±0.13 cm, body weight 22.97±0.75 g).

2. Histological Procedures

Pieces of ovary were fixed in Bouin's solution and then embedded in histoparaffin. The embedded pieces were sectioned at 6 ㎛ and stained with Hansen's hematoxylin-eosin. Some sections were stained with periodic acid Schiff (PAS).

3. Statistics

The results are given as means and their standard errors (SE). Significance between means was determined at the $p < 0.05$ level using Student's *t*-test.

RESULTS

1. Annual Change in GSI

Fig. 2 shows the annual change in the GSI of female *E. merra*. In February, the GSI was less than 1.0. It then increased significantly, peaking (3.41±0.85) in March, before decreasing in April (2.16±0.26) and subsequently remaining below 1.0.

2. Histological Observation of Oocyte Development

Based on the oocyte developmental stages of Takano (1989), development in *E. merra* was divided into the following eight stages:

1) Chromatin-nucleolus Stage (Fig. 3A)

The oocytes are 15 to 20 ㎛ in diameter, and are seen just beneath the surface of the ovigerous lamellae throughout the year. The ooplasm is thin as compared with the oocyte, and a large nucleus occupies most of the oocyte. The nucleus contains

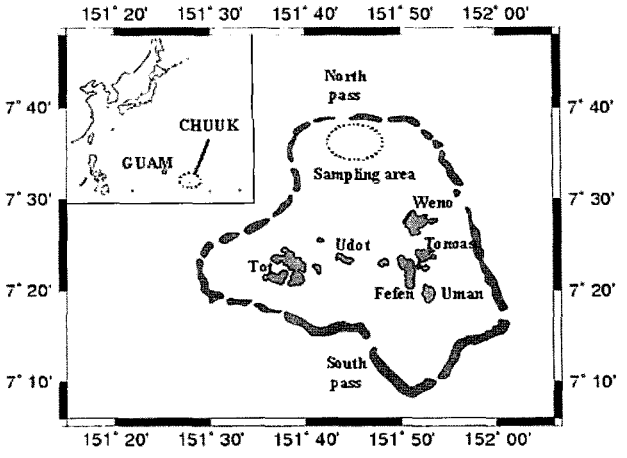

Fig. 1. Sampling areas of *E. merra* in Chuuk Island.

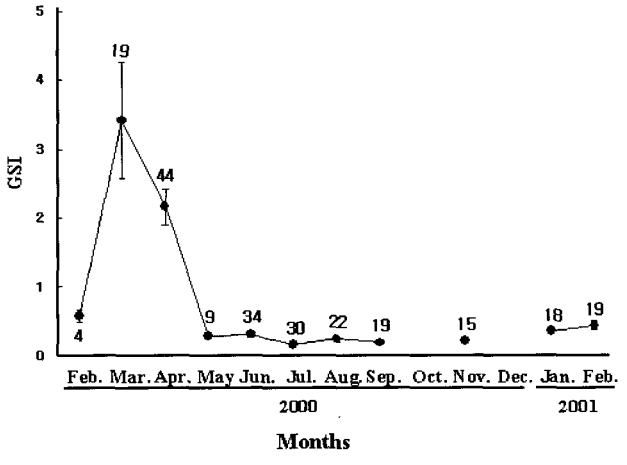

Fig. 2. Seasonal change of gonadosomatic index (GSI) in *E. merra*. Each value represents mean± S.E. Numbers indicate sample sizes.

a chromatin-reticulum accompanying a chromatin-nucleolus.

2) Peri-nucleolus Stage (Fig. 3B, C)

Oocytes at this stage are also observed year-round, and range from 50 to 70 ㎛ in diameter. They are characterized by many nucleoli distributed along the periphery of the nuclear membrane. At the beginning of this stage, the ooplasm stains deeply with hematoxylin (Fig. 3B). As the ooplasm increases in volume with oocyte growth, the yolk nucleus appears in the ooplasm and stains deeply with hematoxylin (Fig. 3C).

3) Oil-droplet Stage (Fig. 3D)

When the oocyte diameter exceeds 100 ㎛, oil-droplets begin to appear in the ooplasm. As the oocyte grows, its affinity with

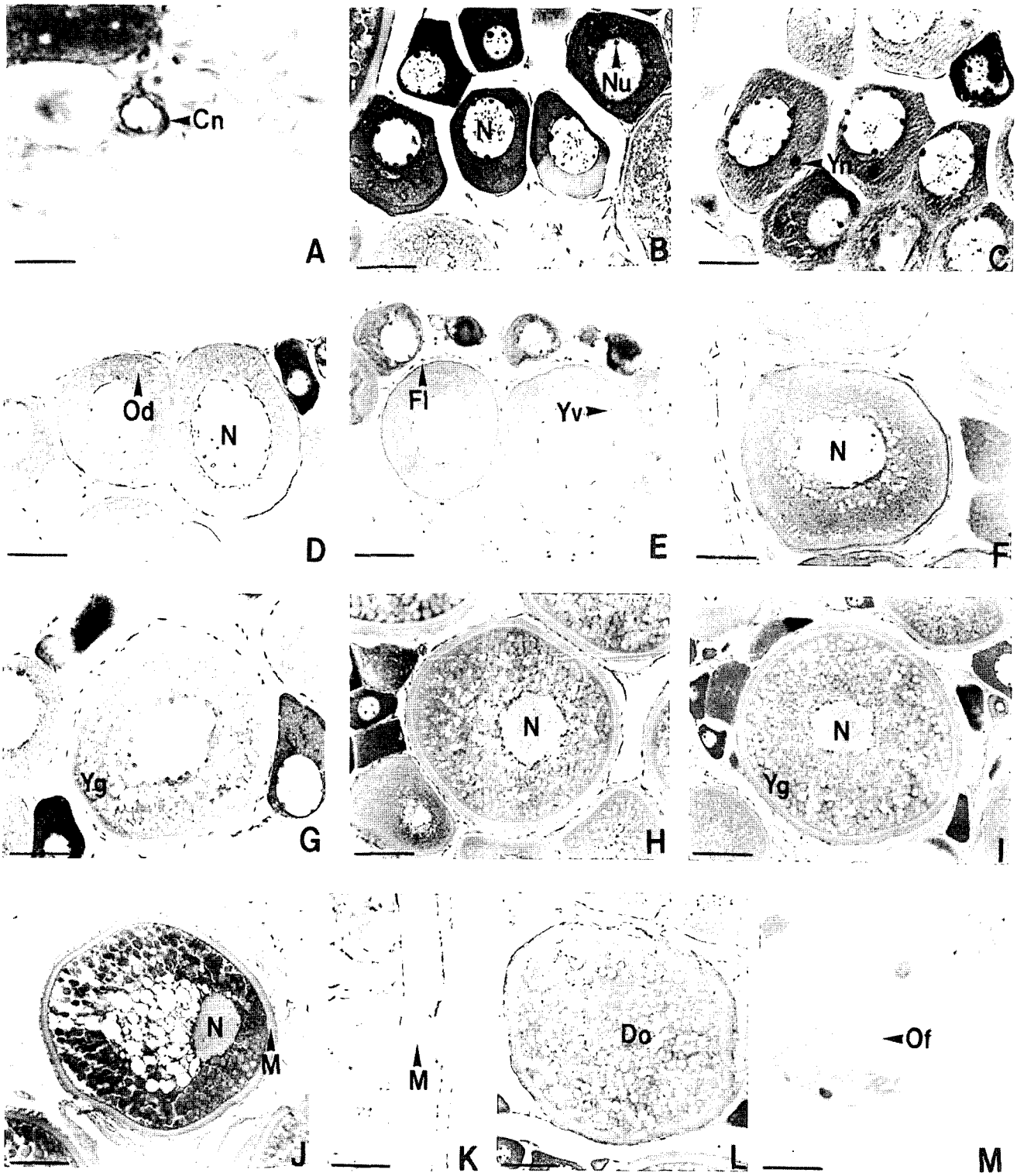

Fig. 3. Histological classification of oocyte developing stage in *E. merra*. A: Chromatin-nucleolus stage (bar=20 ㎛), B: Early perinucleolus stage (bar=50 ㎛), C: Late peri-nucleolus stage (bar=50 ㎛), D: Oil-droplet stage (bar=50 ㎛), E: Yolk vesicle stage with PAS (bar=100 ㎛), F: Yolk vesicle stage (bar=50 ㎛), G: Primary yolk stage (bar=50 ㎛), H: Secondary yolk stage (bar=100 ㎛), I: Tertiary yolk stage (bar=100 ㎛), J: Migration of nucleus stage (bar=100 ㎛), K: Micropyle of a mature oocyte stage (bar=20 ㎛), L: Atretic oocyte (bar=100 ㎛), M: Ovulatory follicle (bar=100 ㎛), Cn: Chromatin-nucleolus oocyte, Do: Degenerating oocyte, Fl: Follicle layer, Od: Oil-droplet, Of: Ovulatory follicle, M: Micropyle, N: Nucleus, Nu: Nucleolus, Yg: yolk globule, Yn: Yolk nucleus, Yv: Yolk vesicle.

hematoxylin gradually decreases. A thin follicle layer is first observed around the oocyte.

4) Yolk Vesicle Stage (Fig. 3E, F)

Oocytes at this stage range from 125 to 150 ㎛ in diameter; PAS-positive yolk vesicles begin to appear in the ooplasm (Fig. 3E). As the oocyte grows, the yolk vesicles rapidly increase in number and volume. The follicle layer is clearly seen and the vitelline envelope is evident (Fig. 3F).

5) Primary Yolk Stage (Fig. 3G)

The diameters of oocytes at this stage range from 150 to 170 ㎛. A round nucleus, about 50 ㎛ in diameter, is located at the center of the oocyte. The yolk vesicles and oil-droplets increase in number and volume, and spread throughout the ooplasm. Yolk globules, which stain strongly with eosin, begin to appear as minute granules in the periphery. The follicle layer thickens. A vitelline envelope more than 5 ㎛ thick is clearly observed as a membrane between the ooplasm and the follicle layer.

6) Secondary Yolk Stage (Fig. 3H)

Oocytes at this stage range from 230 to 260 ㎛. Yolk globules continue to accumulate in the ooplasm. Oil-droplets are scattered in the ooplasm around the nucleus.

7) Tertiary Yolk Stage (Fig. 3I)

Oocytes at this stage range from 320 to 360 ㎛. The oocyte is filled with many large yolk globules about 10 ㎛ in diameter. The nucleus is about 70 ㎛ in diameter and is still located at the center of the oocyte.

8) Migratory Nucleus or Mature Stage (Fig. 3J, K)

The oocyte is about 410 ㎛ in diameter and is filled with many large yolk globules and oil droplets. The nucleus is about 100 ㎛ in diameter and moves toward the micropyle of the oocyte (Fig. 3J). The micropyle is about 7 ㎛ wide and 10 ㎛ deep and is located at the animal pole of the vitelline envelope. The upper cell of the micropyle is about 20 ㎛ and 8 ㎛ in the long and short axes, respectively. The vitelline envelope thickens markedly, reaching 12 ㎛ (Fig. 3K).

The atretic oocyte is almost the same size as at the tertiary yolk stage. However, its features differ, with degradation of the vitelline envelope and yolk globule (Fig. 3L). The follicle layer shrinks after ovulation. The long and short axes of the empty follicle measure about 150 and 100 ㎛, respectively (Fig. 3M).

3. Seasonal Change in Oocyte Composition

Oocytes at each stage were counted under a microscope, and the percentage of each stage in the ovary was determined. Oocytes at the chromatin-nucleolus stage were not analyzed, since they were difficult to count precisely.

Fig. 4 shows the seasonal change in oocyte composition. In February, the ovary contained mostly oocytes at the peri-nucleolus stage, and a few at the oil-droplet and primary yolk stages. In March, oocytes laden with yolk were observed, with mean values of 10.4, 15.5, and 14.6% in the primary, secondary, and tertiary yolk stages, respectively a small number of empty follicles were observed for the first time. In April, most ovaries contained oocytes at the tertiary yolk and mature stages. The percentages of such oocytes increased to 5.2 and 5.0%, respectively. Empty follicles were observed. In May and June, the ovaries contained oocytes at the peri-nucleolus and oil-droplet stages.

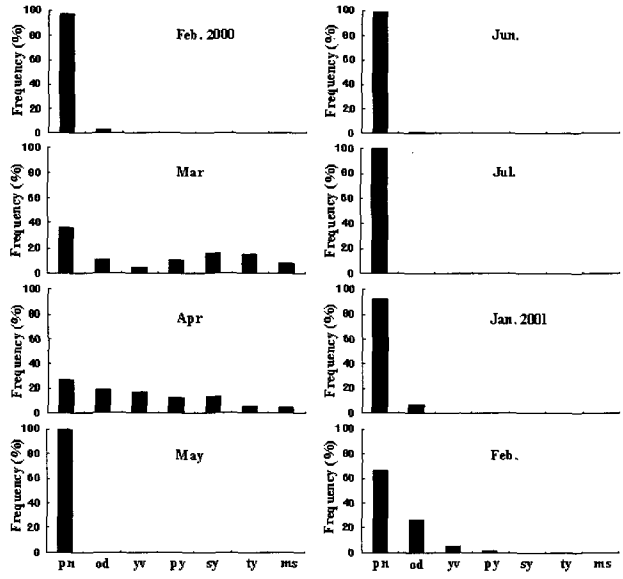

Fig. 4. Frequency distribution of oocyte developing stages from *E. merra* from February 2000 to February 2001 in Chuuk. pn: peri-nucleolus stage, od: oil-droplet stage, yv: yolk vesicle stage, py: primary yolk stage, se: secondary yolk stage, ty: tertiary yolk stage, ms: migratory nucleus or mature stage.

4. Reproductive Cycle

The developmental phases of the ovaries of *E. merra* had four successive stages. The frequencies of oocytes in the developmental phases of *E. merra* are shown in Fig. 5.

1) Early Growing Stage

Individuals in the early growing stage were first observed in January. The number of oocytes in the peri-nucleolus stage in the ovaries gradually increased, and oocytes in the oil globule stage appeared in the ovarian lamellae.

2) Growing Stage

Beginning in February, the GSI gradually increased. The ovaries contained oocytes at the oil globule and yolk globule stages.

3) Mature and Spawning Stage

The GSI peaked in March. Empty follicles were also observed. Individuals at the mature and spawning stages appeared in March and April.

4) Resting and Recovery Stage

The GSI decreased rapidly in May. The ovaries contained mainly immature oocytes and a few atretic oocytes. Individuals at the resting and recovery stages appeared from May to December.

DISCUSSION

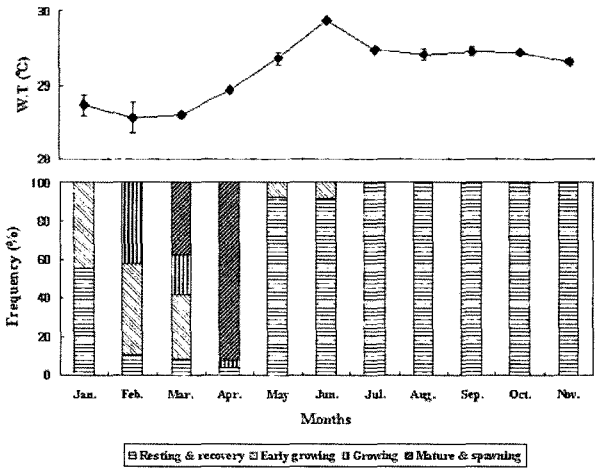

Fig. 5. Frequency of gonadal phases in *E. merra* and the mean seawater temperature from January to November.

In February, the ovaries of *E. merra* contained mainly immature oocytes at the chromatin-nucleolus, peri-nucleolus, and oil droplet stages. In March, many oocytes laden with yolk were scattered among the immature oocytes. Subsequently, oocytes at the tertiary yolk and mature stages occurred in ovaries until April; subsequently no further vitellogenic oocytes were observed in the ovaries. These histological features indicate that reproductive activity in this species in Chuuk coastal waters begins in February and lasts until April. The highest GSI value and the presence of many oocytes at the yolk stage suggest that vitellogenesis in *E. merra* peaks in March.

The reproductive and spawning seasons of various groupers are reported to extend from December through May in the Caribbean (Thresher, 1984) and from April through August in Bermuda (Smith, 1972). *E. morio* spawns from March through July in the Gulf of Mexico, and *E. guttatus* spawns from May to July in Bermuda (Thresher, 1984). We showed that in Chuuk the reproductive season of *E. merra* lasts for at least 2 months, from March to April. By contrast, *E. merra* is reported to spawn during late summer in Tahiti (Randall and Brock, 1960) and from May to August in Okinawa (Lee et al., 2002). These reports reveal local differences in the spawning seasons of tropical groupers. These differences have been explained by the latitudinal differences in the habitats, which affect environmental factors such as water temperature and photoperiod (Rahman et al., 2000).

Thresher (1984) suggested that tropical fish spawn at temperatures below the annual maximum, while populations at higher latitudes spawn at temperatures nearer the local maximum. The water temperature at Chuuk increases gradually, beginning in February, and peaks in June. Therefore, the increasing water temperature could be an important environmental factor in determining the initiation, duration, and termination of the reproductive activity of *E. merra* inhabiting Chuuk coastal waters.

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