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Reproductive Cycle of the Female Grey Mullet, *Mugil cephalus*, on the Coast of Jeju Island, Korea

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제주 연안 숭어, *Mugil cephalus* 암컷의 생식주기

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요 약

숭어의 생식주기를 밝히기 위해 2002년 2월부터 2003년 3월까지 제주도 동남부 연안에서 채집하였고, 난모세포의 발달과정을 조직학적 방법으로 관찰하였다. 난모세포의 발달과정은 GSI 변화와 밀접하였다. GSI는 9월에 급격히 증가하여 12월에 최고 값을 나타내었다. 난소의 조직학적 관찰결과, 염색인기와 주변인기의 난모세포는 연중 관찰되었으며, 8월에는 일부에서 난황을 포함하는 난모세포가 관찰되었다. 대부분의 난모세포가 10월과 12월에는 난황구기 단계의 난모세포로 발달했다. 10월과 12월에 난황구기 난모세포들을 가진 개체의 출현이 가장 높았으며, 잔존여포와 퇴화 난모세포는 2월부터 관찰되었다. 숭어의 주 산란기는 11월에서 1월이고, 난군동기발달형의 난소를 가지며 외양으로 이동하여 산란하는 어류에 속한다.

Key words : Grey mullet, Group synchronous, *Mugil cephalus*, Offshore migration, Reproductive cycle

INTRODUCTION

Coastal environments have changed with the inflow of terrestrial sewage and factory wastes. Endocrine disrupting chemicals (EDCs) might affect the sex differentiation and reproduction of coastal fish. Research has examined the effects of EDCs on the reproduction of fish inhabiting freshwater rivers or lakes (Gray *et al.*, 2002; Jobling *et al.*, 2003), but little research has examined fish in the sea. However, it is difficult to select a coastal fish for study, because

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they can move to a more suitable habitat as pollution worsens.

The grey mullet, *Mugil cephalus*, migrates to feed, moving from seawater to brackish as it grows from fry to adult. The grey mullet is widely distributed throughout the temperate and tropical regions of the Pacific and Atlantic, from about 42°N to 42°S latitude (Martin and Drewry, 1978). Many fish belonging to the family Mugilidae inhabit the coastal waters of Korea, including the grey mullet, *M. cephalus*, fringelip mullet, *Liza haematocheila*, flathead mullet, *M. japonicus*, and eastern keelback mullet, *L. carinata* (Hwang, 1989; Lee and Joo, 1994; Kim and Kim, 1998). The grey mullet is regarded as a commercial fish in a region that has undergone great environmental change. It has highly developed chloride cells that have osmoregulatory capability, so that it can cope with changes in salinity effectively (Nash and Shelhaden, 1980). Therefore, it should be an excellent fish for studying the effects of EDCs on the reproduction of seawater fish.

Extensive research has been conducted on grey mullet in Japan, Taiwan, and Hawaii, including studies of its ecology (Thomson, 1968), distribution (Liu, 1993), spawning (Lee *et al.*, 1992), sex differentiation (Chang *et al.*, 1999), classification (Song, 1981; Senou, 1993), development (Tung, 1973; Walsh *et al.*, 1991), and culture (Eda *et al.*, 1990). Studies in Korea have examined egg and larval development in *Chelon lauvergnii* (Kim *et al.*, 2000), and the rearing ecology (Chu *et al.*, 2000; Chang *et al.*, 2001), and reproductive cycle (Kim and Lee, 1985) of grey mullet.

As a first step to understanding the effects of EDCs on the reproduction of seawater fish, this study investigated the histology of the reproductive cycle and oocyte development in grey mullet inhabiting the coast of Jeju Island.

MATERIALS AND METHODS

Grey mullet were sampled monthly along the southeastern coast of Jeju Island, Korea by fishing, from February 2002 to March 2003. In the laboratory, total length (0.1 cm) and body weight (0.1 g) were measured. The gonad and liver were removed immediately, weighed (0.01 g), and fixed in Bouin's solution. The gonadosomatic index (GSI) was calculated as follows: GSI = GW100/BW, where GW and BW are the gonadal (g) and body (g) weights, respectively. The hepatosomatic index (HSI) was calculated from the liver weight (LW) as follows: HSI = LW100/BW.

For histological observations of the gonads, part of the testis or ovary was fixed in Bouin's solution, dehydrated with ethanol, and embedded in paraffin. The specimens were cross-sectioned at $5 \sim 6$ μm thickness, and stained with Hansen's hematoxylin and 0.5% eosin for light microscopic observation.

To study the environmental change in the sampling areas, the monthly mean water temperatures and photoperiod were obtained from the National Fisheries Research and Development Institute and the Jeju Regional Meteorological Office, respectively.

RESULTS

1. Morphological features of the ovary

Morphologically, the gonads of the grey mullet are paired, elongated structures attached to the body cavity on either side of the dorsal mesentery. The walls of the gonads extended backward to form the gonoducts, which often fuse posteriorly before reaching the genital pore.

The mature ovary is lemon-yellow, and its base has a vascular ramification at the outer walls.

Internally, the ovary is a cystovarian-type ovary with thick walls that are composed of connective and muscular tissues with abundant blood vessels, containing several ovarian cysts between connective tissue partitions.

2. Monthly changes in GSI and HSI

The monthly changes in GSI and HSI of female grey mullet with water temperatures and photoperiod in the sampling areas are shown in Fig. 1.

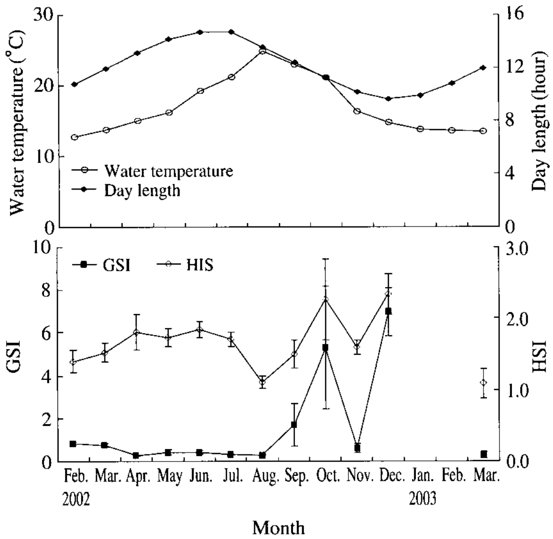

Fig. 1. Monthly changes in GSI and HSI in female *M. cephalus* according to the water temperatures and photoperiod.

The GSI remained relatively low from February (0.83±0.10) to August (0.29±0.06), and then increased rapidly in September (1.70±1.01) when the water temperature and daylength began to decreased to 23°C and 12.43D, respectively, to peak (5.32±2.87) in October. The GSI decreased dramatically in November (0.61±0.20), but exhibited a second maximum (6.97±1.14) in December when daylength was minimal at 9.62D.

The HSI of female fish in the maturing and spawning periods roughly paralleled the monthly fluctuation in the GSI.

3. Seasonal Change in Oocyte Composition

Fig. 2 shows the seasonal change in the oocyte composition. The rate of atretic oocytes was highest in February (1.9%), and March (0.9%), while the oocytes in the oil-droplet stage began to appear in August, increased to 8.3% by October, and then began to decrease in December with oocytes development. Oocytes in the yolk globule stage were seen in September (6.4%), and peaked in December (17.9 %). Mature oocytes began to appear in September (6.3%), and also peaked in December (53.6%).

4. Fecundity

The total fecundity according to total length and weight was examined in six mature female fish. The fish with the maximum fecundity was 64.5 cm in total length weighed 2,453 g, and had 3,715,693 eggs. The fish with the minimum fecundity was 51.2 cm in total length, weighed 1,247.5 g, and had 2,179,001 eggs.

5. Histological observation of oocyte development

Oocytes of grey mullet were divided into six developmental stages.

1) Chromatin-nucleolus stage

The smallest oocytes of about 10 μm in diameter were found in just beneath the surface of 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 a chromatin-reticulum accompanying a chromatin-nucleolus.

2) Peri-nucleolus stage

Oocytes at this stage are observed year-round, and ranged from 20 to 100 μm 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. As the ooplasm increases in volume with oocyte growth, the yolk nucleus appears in the ooplasm and stains deeply with hematoxylin.

3) Oil-droplet stage

The size of oocytes is ranged from 100 to 150 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

The size of oocytes at this stage is ranged from 150

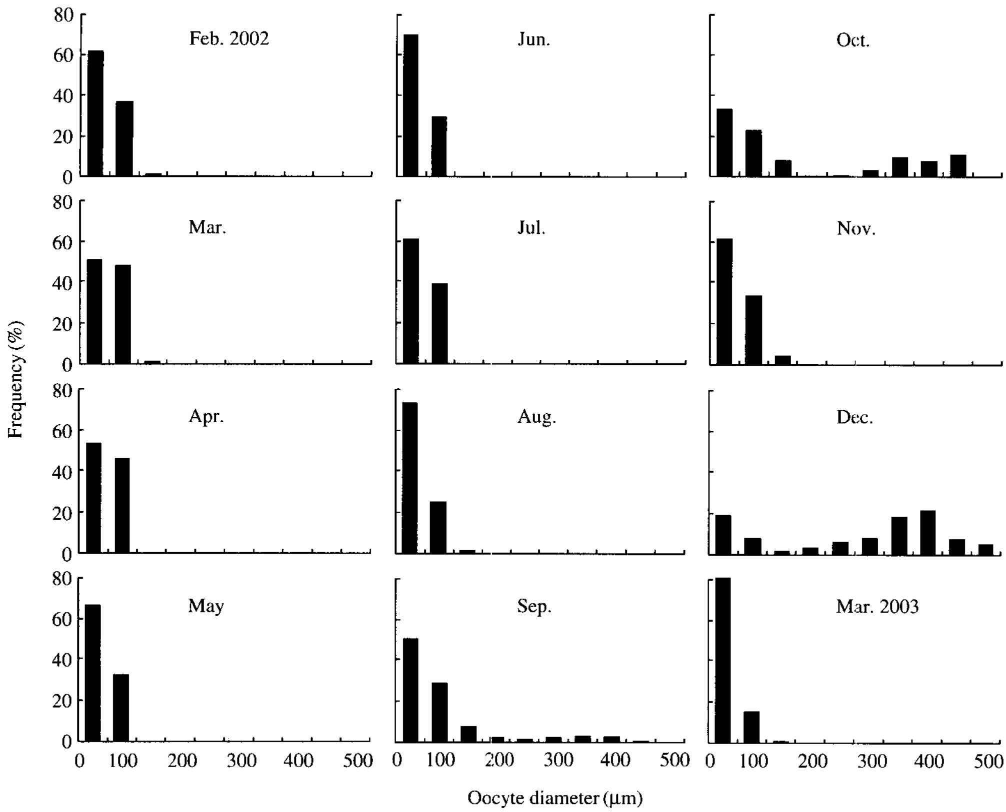

Fig. 2. Monthly change in oocyte diameter in the ovary of female *M. cephalus* from February 2002 to March 2003.

to 180 μm. A round nucleus, about 50 μm 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 μm thick is clearly observed as a membrane between the ooplasm and the follicle layer.

5) Secondary yolk stage

Oocytes at this stage are ranged from 200 to 270 μm. Yolk globules continue to accumulate in the ooplasm. Oil-droplets are scattered in the ooplasm around the nucleus.

6) Tertiary yolk stage

As yolk globules gradually increased in number and size, the oocytes became larger ranging from 300 to 480 μm in diameter. The entire cytoplasm was filled with many yolk globules and oil-droplets which scattered in cytoplasm.

6. Reproductive Cycle

Based on the histological observations of gonad development and monthly fluctuation in GSI, the grey mullet ovaries undergo five successive stages.

The frequency distribution of ovary developmental stage is shown in Fig. 3.

1) Early Growing Stage

Individuals in the early growing stage were first observed in April. The number of oocytes in the peri-nucleolus stage ranging from 20 to 100 μm in diameter in the ovaries increased gradually, and oocytes in the oil droplet stage ranging from 100 to 150 μm in diameter appeared in the ovarian lamellae

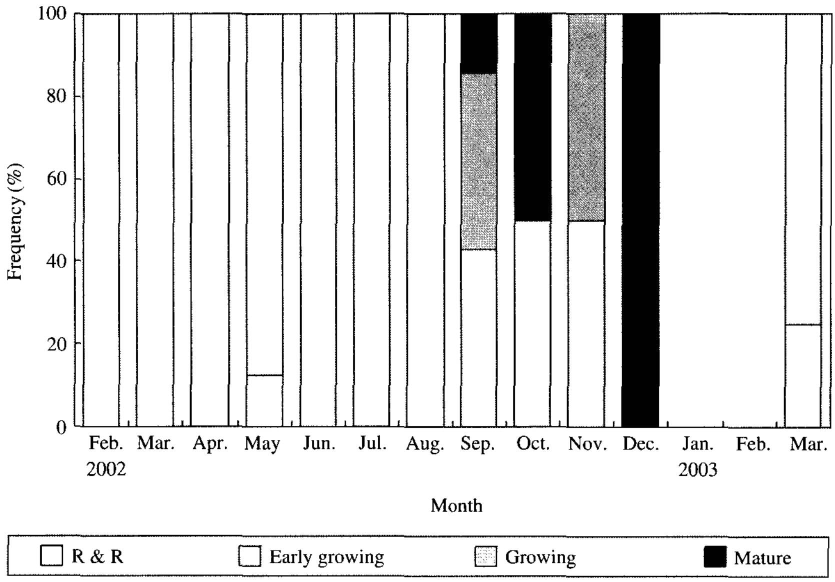

Fig. 3. The frequency distribution of ovary developmental stage of *M. cephalus* from February 2002 to March 2003.

(Fig. 4A).

2) Growing Stage

In September, the ovary accumulated oocytes in the yolk globule stage with a granular yolk globule ranging from 150 to 300 μm in diameter, including those in the oil-droplet stage (Fig. 4B).

3) Mature Stage

The GSI peaked in October and December. Individuals had mature oocytes stages ranging from 300 to 480 μm in diameter with a big oil-droplet (Fig. 4C).

4) Spawning Stage

There was individual variation in the migration time, and fish migrate offshore between November and January to spawn, so there was no single population in the spawning stage.

5) Resting and Recovery Stage

The GSI decreased rapidly in February. The ovaries contained mainly immature oocytes and a few ovulatory follicles. Individuals in this stage appeared from February to March (Fig. 4D).

DISCUSSION

Water temperature and photoperiod exert the strongest effects on the reproductive rhythm of fish (Asahina and Hanyu, 1983; Razani and Hanyu, 1986; Santos *et al.*, 1986; Bromage *et al.*, 2001). The grey mullet gonads began to develop in September, when the water temperature began to decrease, and the GSI increased rapidly to 1.70±1.01, and was maximal at 6.97±1.14 in December when the daylength was shortest at 9.62D. The HSI of female fish in the maturing and spawning periods roughly paralleled the monthly fluctuation in the GSI. The HSI was relatively high in October and December, when vitellogenesis was confirmed, indicating that vitellogenin was synthesized in the liver during this period. These results suggest that the grey mullet is a winter-spawning fish, which characteristically spawns between November and January, when the photoperiod is short and water temperature low. Other winter-spawning fish inhabiting the coast of Jeju Island include the Japanese seabass, *Lateolabrax japonicus* (Kang *et al.*, 2001), oblong rockfish, *Sebastes oblongus* (Chang *et*

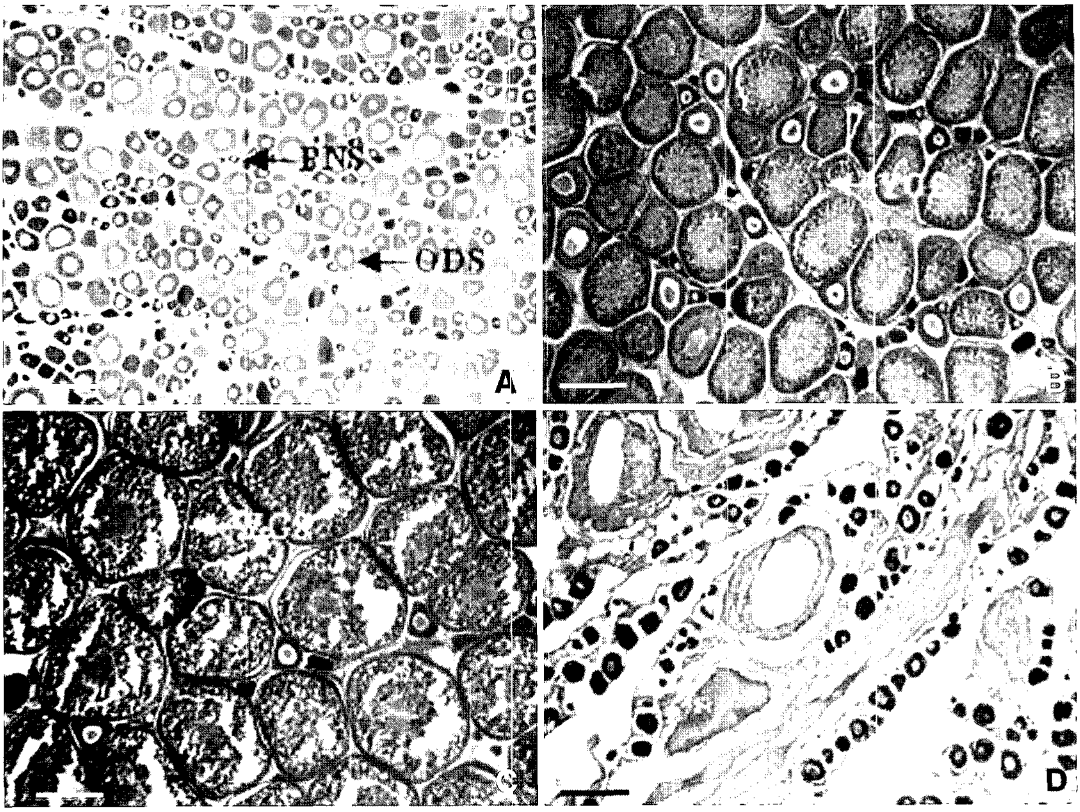

Fig. 4. Photomicrographs of ovarian development of *M. cephalus*. A: Early growing stage, B: Growing stage, C: Mature stage, D: Resting and recovery stage. ODS: oil-droplet stage, OF: ovulatory follicle, PNS: peri-nucleolus stage, YGS: yolk globule stage. Scale bar = 200 μm.

al., 1995), and marbled rockfish, *S. marmoratus* (Chung *et al.*, 1998).

Group-synchronous type fish are subdivided into two groups that differ according to the developmental stage within the ovary; a mature or nearly mature oocyte group and an undeveloped oocyte group. The undeveloped oocyte group does not contribute during the spawning season. Fish of this type usually spawn once a year and several times in a lifetime. The spawning season is relatively short (Wallace and Selman, 1981; de Vlaming, 1983). Japanese seabass, *L. japonicus* (Kang *et al.*, 2001), rabbitfish, *Siganus canaliculatus* (Hwang, 1999), goldeye rockfish, *S. thompsoni* (Huh *et al.*, 1998), and smallmouth scorpionfish, *Scorpaena miostoma* (Huh *et al.*, 1997) belong to this type. The grey mullet is a group-synchronous spawner, and two groups of oocytes are seen in the ovary near the spawning period. It is thought to spawn once a year; the developmental stage of the mature oocytes is not separated during the short time after activities begin within the ovary, but oocytes pass through each stage consecutively.

The fecundity of fish generally increases with total length and weight, as was observed in the grey mullet.

Both coastal and oceanic fishes perform spawning migration, usually migrating from offshore to onshore, since the coast has more food for juvenile and fry, making it more suitable for their development and growth. Fish that migrate onshore include the seabass, *L. japonicus* (Kang *et al.*, 2001) and tiger puffer, *Takifugu rubripes* (Tokai *et al.*, 1993). By contrast, the grey mullet migrates offshore to spawn (Brusle, 1981; Sostoa, 1983; Cambrony, 1984), like the North Sea plaice, *Pleuronectes platessa* (Bromley, 2000), southern flounder, *Paralichthys lethostigma* (Moustakes *et al.*, 2004) and Atlantic menhaden, *Brevoortia tyrannus* (Stegmann and Yoder, 1996). Low water temperature cause immature grey mullet to migrate offshore to pass the winter, while salinity causes mature fish to migrate offshore in the spawning season (Cardona, 2000; Tamaru *et al.*, 1994). In this study, mature individuals were collected in October and December, but because the fish migrate offshore to spawn, it was difficult to collect mature fish on the coast in November and January, during the presumed spawning period.

This study investigated the oogenesis, reproductive cycle, and fecundity of grey mullet inhabiting the coast of Jeju Island. This work will serve as the base for a comparative study of oogenesis in grey mullet inhabiting the coastal waters and brackish industrial regions along the coast of Jeju Island and the Korean peninsula.

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