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Gonad Ontogeny in Relation to Somatic Growth in the Brown Croaker *Miichthys miiuy* (Basilewsky)

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Sex differentiation of the brown croaker *Miichthys miiuy* (Basilewsky) is described from hatching to the 120th day post-hatching (dph) (water temperature 24°C). Primordial germ cells (PGCs) were observed on the 20th dph (10.4 mm total length (TL), 0.14 g body weight (BW), and began to protrude into the peritoneal cavity from the 40th dph (19.4 mm TL, 0.39 g BW). On the 65th dph (31.3 mm TL, 0.93 g BW, 1,560 D° (degree-days)), initial ovarian differentiation was identified by the PGCs with condensed chromatin, and their transformation into meiotic oocytes. By the 120th dph (4.60 mm TL, 1.38 g BW, 2,880 D°), the oocytes were in the perinucleolus stage and had increased from 20 to 40 μm in diameter. While ovaries gradually grew after sex was differentiated, testes continued to multiply from the 65th dph. On the 80th dph (37.9 mm TL, 1.39 g BW, 1,920 D°), the beginning of testis lobule formation was indicated by the occurrence of spermatogonial cysts enveloped by somatic cells in some of the testes. On the 120th dph, the testis lobules of some of the fish contained all germ cell stages through to the spermatocytes. Therefore, the sex differentiation type of the brown croaker is identified as gonochoristic.

Key words : differentiated gonochorism, gonadal development, growth, *Miichthys miiuy*

Introduction

Sexual patterns in teleosts are diverse and include gonochorism, in which individuals function as either females or males throughout their lifetimes, and hermaphroditism, in which individuals function as both sexes, either simultaneously or sequentially (Atz, 1964; Park *et al.*, 2004).

The sexual pattern of the brown croaker *Miichthys miiuy* (Basilewsky) is not fully understood. This fish is the most common member of the Sciaenidae in Korea, where it is an important commercial component of the longline fishery. Brown croakers occur in the Yellow Sea, the Southern Sea of Korea, the Southwest Sea of Japan, and the South China Sea (Choi *et al.*, 2002). Our objective is to describe early gonadal development of the species in order to determine the sex-

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ual pattern.

Materials and Methods

The experimental animals were laboratory-bred offspring raised in the Gyeongsangnam-do Fisheries Resources and Research Institute, Korea, following standard methods for artificial culture. Newly hatched larvae were kept at an incubation temperature of 24±1°C. Animals (n =10) were sampled at 5-day intervals from hatching to the 100th day post-hatching (dph), and at 10-day intervals from the 110th to 180th dph. Total body weights and lengths of 10 freshly sampled larvae at different developmental stages were measured to the nearest 0.01 g and 0.1 mm, respectively; prior to measurements, the larvae were anesthetized with 300 ppm lidocaine-HCL/ $NaHCO_3$ at 25°C. Growth in total length and body weight were examined with the von Bertalanffy and Gompertz equations, respectively (Gompertz, 1925; von Bertalanffy, 1938).

After measuring weights and lengths, samples were fixed in 10% neutral formalin for histological observations. After fixation in Bouin's fixative for 24 h, specimens (excluding the head and caudal region) were prepared for sectioning by routine dehydration and paraffin embedding procedures. Cross sections (4～6 μm thick) were stained with Mayer's hematoxylin and eosin phloxine B solution, examined, and photographed under light microscopy.

Results and Discussion

Figure 1 shows the trends in total length and body weight of fish from hatching to the 180th dph. Total length (TL) increases indicate continuous growth of fish, as described by the von Bertalanffy growth expression $TL=1.5409t^{0.7111}$ (R^2 =0.96). Continuous growth in body weight (BW) also occurred, as described by the Gompertz expression $BW=0.0027t^{1.4036}$ (R^2=0.98). Growth in total length accelerated until 180th dph.

On the 20th dph (10.4 mm TL, 0.14 g BW, 480 D° (degree-days)), primordial germ cells (PGCs) were clearly visible between the peritoneal wall and gut in the posterior trunk region (Fig. 2a). On the 40th dph (19.4 mm TL, 0.39 g BW, 960 D°), the primordial germ cells began to protrude into the peritoneal cavity. Each was enclosed

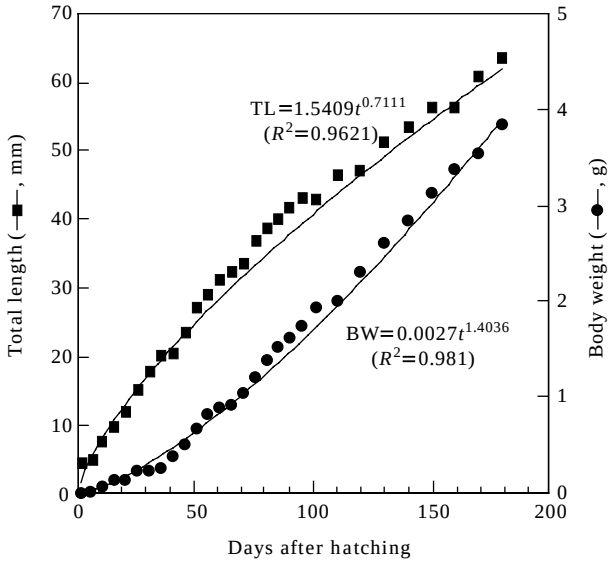

Fig. 1. Growth curves in total length (TL) and body weight (BW) of the brown croaker, *Miichthys miiuy* from hatching to 180th day post-hatch. *t*: time.

separately by somatic cells in the future gonadal area (Fig. 2b). The PGCs were identified easily by their more or less irregular shape, their size (3～4 μm in diameter), and their enclosure in loose connective tissue. PGCs developed along the peritoneal wall of the coelomic cavity at the site of the future gonadal ridges. During the larval period, the gonadal anlarge gradually arose from the PGCs and surrounding somatic cells (Yamamoto, 1969; Timmermans, 1987; Nagai *et al.*, 2001).

PGCs containing condensed chromatin and oocytes (4～5 μm in diameter) in meiotic prophase were present on the 50th dph (24.2 mm TL, 0.64 g BW, 1,200 D°) . The PGCs and oocytes were enclosed by somatic cells. On the 65th dph (31.3 mm TL, 0.93 g BW, 1,560 D°), we observed numerous oocytes in meiotic prophase and with meiotic figures. The oocytes were in the chromatin-nucleolus stage. The ovary was beginning to fill with blood vessels and the endoovarian canal (Fig. 2c). This early-stage differentiated ovary became apparent on the gut, adjacent to the liver or embedded within it. In most cases, PGCs appeared to migrate directly to gonad tissues. This is in contrast to the Caspian Sea sturgeon PGCs, which migrate to other organs, such as the liver (Romanov and Altuf'ev, 1993). By the 120th dph (4.60 mm TL, 1.38 g BW, 2,880 D°), the oocytes were in the peri-nucleolus stage and had increased from

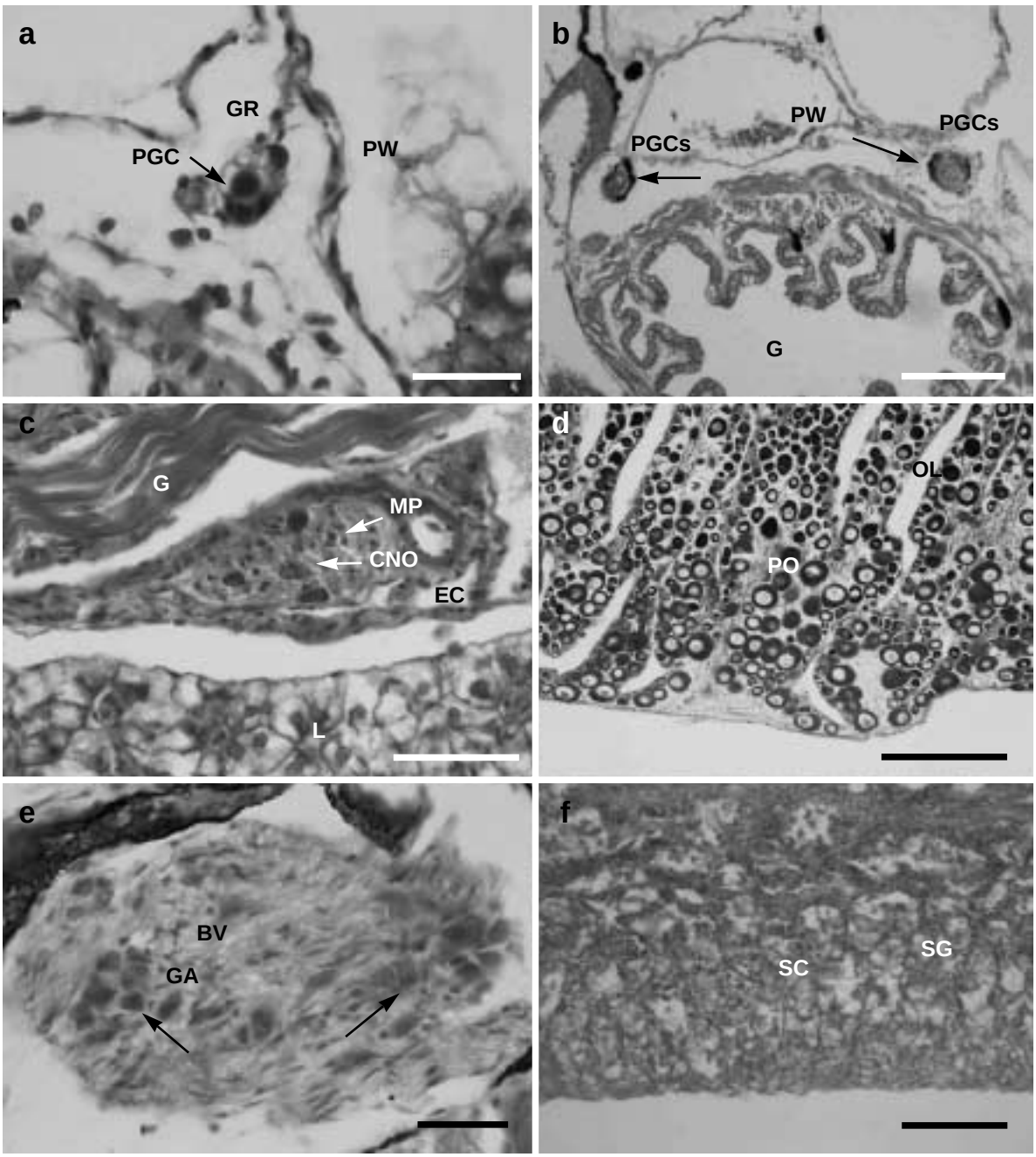

Fig. 2. Histological sections (H-E stain) of brown croaker, *Miichthys miiuy* gonads showing successive stages of gonadal differentiation. (a) Gonad on the 20th day post-hatch (dph). The gonad consists of a genital rideg adjacent to the swimbladder and contains primordial germ cell (scale bar, 10 µm). (b) Undifferentiated gonad on the 40th dph. with clutches of primordial germ cells (arrowed) protruding into the peritoneal cavity (scale bar, 100 µm). (c) Early differentiated ovary on the 65th dph. Note the presence of oocyte in the chromatin-nucleolus stage. Meiotic figures become common (scale bar, 10 µm). (d) Completely differentiated ovary on the 120th dph, with, primary oocyte and a well developed ovarian lamellae (scale bar, 200 µm). (e) Undifferentiated presumptive male gonads on the 80th dph showing a larger part of the gonad is occupied by stromal cells. In addition, groups of gonial cells are present and they are arranged in cyst-like manner (arrows) (scale bar, 10 µm). (f) Tests on the 120th dph, showing cysts filled with spermatogonia as well as spermatocytes (scale bar, 50 µm). BV, blood vessel; CNO, oocyte in chromatin-nucleolus stage; G, gut; GA, gonial cell; GC, germ cell; GR, genital ridge; EC, endoovarian canal; L, liver; MP, meiotic prophase; OL, ovarian lamella; PGC, primordial germ cell; PO, primary oocyte; PW, peritoneal wall; SC, spermatocyte; SG, spermatogonia.

20 to 40 μm in diameter (Fig. 2d).

Development in the male reproductive system was rather different. The PGCs (spermatogonia) of the presumptive testes did not enter meiosis until the 80th to 120th dph. Through the 65th dph, spermatogonia remained quiescent, and then gradually increased in number through mitotic divisions. In contrast to the developing ovaries, somatic cells were scattered throughout the gonads from the earliest stages. On the 80th dph (37.9 mm TL, 1.39 g BW, 1,920 D°), the beginning of testis lobule formation was indicated by the occurrence of spermatogonial cysts enveloped by somatic cells in some of the testes (Fig. 2e). This was more apparent on the 120th dph when the testes contained many lobules, each with a clear central lumen (Fig. 2f). At this time, the testis lobules of some of the fish contained all germ cell stages through to the spermatocytes. PGC differentiation in males occurred up to 15 days later than in females (which started on the 65th dph). Ovaries were clearly discernable under light microscopy between the 65th and 120th dph. In constrast, definitive testes were not identifiable by their PGCs until between 80th and 120th dph. This developmental difference between the sexes is common among gonochoristic fish (Yamamoto, 1969; Nakamura *et al.*, 1998; Park *et al.*, 2004).

Our observations show that the brown croaker is a differentiated gonochoristic teleost. Ours is one of few studies to present detailed simultaneous chronological descriptions of the gonadogenesis in this species. The work establishes a protocol for sex reversal, which may be useful for laboratory studies of sexual expression and for potential aquaculture exploitation of the brown croaker.

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민어, *Miichtys miiuy* (Basilewsky)의 성장과 연관된 생식소 발달

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부화부터 부화 후 120일까지 민어, *Miichthys miiuy* (Basilewsky)의 성분화를 조사하였다. 시원세포는 부화 후 20일 (전장 10.4 mm, 체중 0.14 g)에 출현하여 부화 후 40일 (전장 19.4 mm, 체중 0.39 g)에 복강으로 이동하기 시작하였다. 부화 후 65일 (전장 31.3 mm, 체중 0.93 g, 1,560 D° (적산온도))에 응축된 염색질 상태인 시원세포는 감수분열을 보여 난소로의 분화가 확인되었다. 부화후 120일 (전장 4.60 mm, 체중 1.38 g, 2,880 D°)에 난모세포는 주변인기 단계로 직경이 20～40 μm로 증가하였다. 성분화후 난모세포는 크기 증가를 보인 반면, 정소는 부화 후 65일부터 증식하기 시작하였다. 부화 후 80일 (전장 37.9 mm, 체중 1.39 g, 1,920 D°)에 정소 체세포에 싸여진 정원세포 낭포의 출현과 정소 소엽 형성이 시작되었다. 부화 후 120일에 정소 소엽에는 시원세포와 정모세포가 존재하였다. 본 연구 결과 민어의 성분화 양상은 분화형자웅이체이다.