Sexual Maturity and Early Life History of the Mudskipper Scartelaos gigas (Pisces, Gobiidae): Implications for Conservation

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Abstract
Scartelaos gigas is an amphibious mudskipper species that inhabits mud flats in Korea, China, and Taiwan. This fish is at risk of extinction because of its very restricted habitat and overexploitation. Information about this fish’s reproductive characteristics is needed for species conservation. The sexual maturity and early life history of S. gigas were investigated through histological methods and direct observation of eggs in the wild, respectively. In total, 560 individuals of S. gigas were collected with the aid of fishermen from March 2003 to October 2003 at Jung-do Island, southwest Korea. Through microscopic observations of gonadal development, it was determined that S. gigas of both sexes were immature in April, but began to reach maturity in May, and were then fully mature by June, which was maintained until July. In August, some female fish developed early oocytes, but by September oocytes were observed to have degenerated and had been absorbed. Spawned eggs were elliptical and had an average size of 1.37 mm (long axis) by 0.69 mm (short axis). The newly hatched larvae (3.03 mm total length, TL) had an open mouth and anus, two melanophores near the anus, and one large melanophore between the 18th and 19th myomeres. The larvae (3.18 mm TL) showed absorption of the yolk and oil globule within 5 days after hatching and became prelarvae. This species should be considered vulnerable or conservation-dependent, and thus parental fish need to be protected from fishermen during the main spawning season (June).

Key words: Scartelaos gigas, Sexual maturity, Early life history, Conservation, Gobiidae.

Introduction
Scartelaos gigas (Perciformes, Gobiidae), a kind of mudskipper goby, is restricted to the mud flats of Korea, China, and Taiwan (Murdy, 1989; Lin et al., 1994; Randall and Lim, 2000; Park et al., 2008). In Taiwan, S. gigias is considered in danger of becoming extinct (Dr. Shao, personal communication). This fish may be at a high risk of extinction because of its very restricted habitat compared with Boleophthalmus pectinirostris (Park et al., 2008). Regarding habitat characteristics, Kim et al. (in preparation) documented that S. gigas prefers to live in mud flats with more than 99% mud, but B. pectinirostris prefers less than 95% mud; accordingly, the former shows a very restricted distribution. Additionally, habitat destruction by indiscreet coastal development by government and overexploitation by fishermen can hasten the extinction of these species (Park et al., 2008). Many studies have focused on the more widely distributed mudskipper, B. pectinirostris,
including a series of studies on its aquaculture in Japan during
the late 1980s (Koga et al., 1989a, 1989b, 1989c; Noda and
Koga, 1990a, 1990b; Yuzuriha and Koga, 1990; Yuzuriha et
al., 1990; Koga and Baba, 1991; Washio et al., 1991). There
have also been many reports on B. pectinirostris in Korea,
including studies of its oocyte maturation (Chung et al., 1989),
sexual maturation (Chung et al., 1991), age and growth (Jeong
et al., 2004), growth of age-0 fish (Kim and Jeong, 2007), and
habitat and spawning (Jeong et al., 2010). However, there is no
reported study of S. gigas except a single study on its age and
growth (Park et al., 2008). This dearth of studies on S. gigas
is probably attributable to its very limited habitat compared
with that of B. pectinirostris, as mentioned above. However,
S. gigas and B. pectinirostris are both widely consumed by
humans, and thus the populations of both these fishes may be
decreased through overfishing.

From the viewpoint of species conservation and fisheries
management, the growth and reproductive ecology of S. gigas
are of primary interest. Thus, the present study investigated
the sexual maturity and early life history of S. gigas in Ko-
rea to gain valuable information for species conservation and
management.

Materials and Methods

Sample collection and measurements

In total, 50-100 individuals of S. gigas were collected bi-
weekly by the aid of fishermen using a fishing pole and hook
from March 2003 to October 2003 at Jung-do Island mud
flats in southwestern Korea (Table 1, Fig. 1). The fishes’ total
length (mm) and body weight (g) were measured, and they
were dissected to determine the sex ratio (female / pooled)
and sexual maturity. The χ²-test was used to detect differences in
the sex ratio (Minitab Program).

Histological examination

The extracted gonads were fixed with Bouin’s solu-
tion, then embedded in paraffin, sectioned at a thickness of
3-5 µm, and stained with Mayer’s hematoxylin and 0.5% eo-
sin (H&E). Assessment of the developmental stage of the go-
ndas and their relative stages was based on that of Yasutake
and Wales (1983). The gonads were observed under an optical
microscope, and gonadal development was classified into
five phases: immature, mature, ripe, spent, and degenerated.

Spawning characteristics and early life history

To examine the habitat structure of S. gigas, we mixed
Komaica solution (PT380; Geumgang-koryo Co., Korea)
and hardener and used it to fill a burrow where S. gigas were
known to spawn. After 2 to 3 h, the mud around the burrow
was excavated, following the method of Koga and Noda
(1992). Egg masses protected by a parent in the spawning bur-
row were collected with the aid of fishermen and transferred
to the laboratory. Egg diameter and larval size were measured
using the Image Pro Plus program (version 4.5; Cybernetics,
USA) with a stereomicroscope (SZH10, Olympus, Japan).
The shapes of embryos in the eggs and larvae after hatch-
ing were observed and photographed using a digital camera
attached to the stereomicroscope. The hatched larvae were
reared in a small water tank at a water temperature of 20°C
for 5 days. The terminology used for eggs and larvae followed
that of Russell (1976) and Okiyama (1988).

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Results

Sexual maturity

The biweekly variations in the gonadosomatic index (GSI) of *S. gigas* are shown in Fig. 2. GSI in females was low until May 12, but increased abruptly on May 28, reached a maximum during June 10 to July 15, and decreased suddenly on July 31. The mean values of GSI in females were greater than 6.0 between May 28 and July 15. Similarly, in males, GSI was low until May 12, but increased rapidly and reached a maximum on May 28, which was maintained until July 7, after which GSI decreased gradually. The mean values of GSI in males were greater than 0.1 between May 28 and July 7 (Fig. 2).

The *S. gigas* testis is typically an elongated paired organ with a threadlike shape found in the dorsal part of the body cavity and attached to the air bladder anteriorly and to the urogenital pore posteriorly. Spermatogonia were found in the testicular tubule of an 18.1-cm total length (TL) specimen collected in April 2003 (Fig 3A). Germ cells were observed in the testicular cyst, which was densely stained with

**Fig. 2.** Monthly change in gonadosomatic index (GSI) of *Scartelaos gigas*. Open circles indicate mean and bars indicate minimum and maximum.

**Fig. 3.** Histological sections showing developmental stages of male *Scartelaos gigas*. (A) Immature stage, 30, April, (B) Mature stage, 10, June, (C) Ripe stage, 25, June, (D) Just after spent stage, 7, July, (E) Spent and degenerative stage, 21, September. Ps, primary spermatocytes; Sd, spermatids; Ss, secondary spermatocytes; Sl, seminiferous lobules; Sz, spermatozoon; Tl, testicular lobule.
and oil droplets only in the cortical layer of the cytoplasm, and mature eggs containing a fused yolk granule and without a visible nucleus were observed in a 13.7-cm TL specimen collected in June 2003 (Fig. 4B). Mature oocytes of 200-400 µm in diameter with a homogeneous appearance were found in a 17.1-cm TL specimen collected in June 2003 (Fig. 4C). Many oil droplets were seen in 150-370 µm oocytes in a 13.7-cm TL specimen collected in July 2003 (Fig. 4D). Oocytes and oogonia were seen along with ovarian lamellae in a 16.9-cm TL specimen collected in August 2003 (Fig. 4E). No mature eggs, but 20-30 µm resting early oocytes were observed in a 19.2-cm TL specimen collected in September 2003 (Fig. 4F). These results suggest that the gonadal tissue of female *S. gigas* is ripe from June until mid-July.

The mean sex ratio was 53:47 (female:male); in particular, the ratio of males was significantly higher in October ($P = 0.006$) (Fig. 5), suggesting that the males feed more actively than females just before hibernation. The smallest female specimen containing mature gonads had a TL of 11.6 cm, suggesting that females of at least 11 cm TL are able to spawn.

Fig. 4. Histological sections showing developmental stages of female *Scartelaos gigas*. (A) Immature stage, 30, April, (B) Mature stage, 25, June, (C) Ripe stage, 10, June, (D) Just after spent stage, 15, July, (E, F) Degenerative and resting stage, 13, August (E) and 21, September (F). N, nucleus; No, nucleolus; Oc, ovarian cavity; Od, oil droplet; Og, oogonia; Ol, ovarian lamella; Oo, oocytes; Po, primary oocytes; Yg, yolk globule.
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Yolk were observed. The oil globule was located in the anterior portion of the yolk. There was one melanophore on both the dorsal and ventral portions of the anus and there was one large branch-shaped melanophore on the ventral contour between the 18th and 19th myomeres (Fig. 7B). Three days after hatching, larvae had a TL of 3.05–3.26 mm (mean, 3.17 mm; n = 9) and the preanal length was 45.9% of the TL. The melanophore on the ventral contour of the mid-tail was large, and new melanophores were found on the peritoneum (Fig. 7C). At 5 days after hatching, larvae had a TL of 3.13–3.23 mm (mean, 3.18 mm; n = 7) and the preanal length was 45.4% of the TL. The egg yolk was completely absorbed and only a small oil globule remained. Three or four melanophores were newly visible on the ventral abdomen (Fig. 7D). The amount of egg yolk declined rapidly over time and it had completely disappeared by the fifth day after hatching. However, the oil globule declined in size relatively.

Early life history

One male specimen was found protecting eggs in the spawning burrow. The spawning burrow was oval and 20–25 cm in length, allowing air to exit the burrow (Fig. 6). The eggs adhered to the upper surface of the spawning burrow, attached by numerous fine filaments (Fig. 7A and 7B).

The spawned eggs had an elliptical shape, with a long axis of 1.23-1.48 mm (1.37 ± 0.07 mm, n = 17) and a short axis 0.67-0.71 mm (0.69 ± 0.02 mm, n = 17); thus, the long axis was approximately two times longer than the short axis. There were no melanophores in embryos just after collection, and one large oil globule and one small oil globule were observed (Fig. 7A).

Hatched larvae had a TL of 2.58–3.24 mm (mean, 3.03; n = 12) and their preanal length was about 45.7% of TL. The mouth and anus were open and an air bladder and a large egg yolk were observed. The oil globule was located in the anterior portion of the yolk. There was one melanophore on both the dorsal and ventral portions of the anus and there was one large branch-shaped melanophore on the ventral contour between the 18th and 19th myomeres (Fig. 7B). Three days after hatching, larvae had a TL of 3.05-3.26 mm (mean, 3.17 mm; n = 9) and the preanal length was 45.9% of the TL. The melanophore on the ventral contour of the mid-tail was large, and new melanophores were found on the peritoneum (Fig. 7C). At 5 days after hatching, larvae had a TL of 3.13-3.23 mm (mean, 3.18 mm; n = 7) and the preanal length was 45.4% of the TL, similar to that at 3 days after hatching. The egg yolk was completely absorbed and only a small oil globule remained. Three or four melanophores were newly visible on the ventral abdomen (Fig. 7D). The amount of egg yolk declined rapidly over time and it had completely disappeared by the fifth day after hatching. However, the oil globule declined in size relatively.

Fig. 5. Monthly change of sex ratio (female/pooled) of *Scartelaos gigas*.

Fig. 6. Habitat cave of *Scartelaos gigas*. (A) Entrance, (B) Real passage, (C) Spawning cave, (D) Camouflage passage, (E) Mud surface. Egg mass of *S. gigas* was found in the upper layer of spawning cave along with one male protecting egg mass. Sometimes both one male and one female were found in the spawning cave during spawning season. Fishermen obstructed escape of *S. gigas* by stepping on real passage, then caught *S. gigas* through entrance by hand.

Fig. 7. Eggs and larval development of *Scartelaos gigas*. (A) Egg mass, (B) Embryonic stage, (C) Just-hatched larva, (D) Three days larva after hatching, (E) Five days larva after hatching.
slowly and could still be seen on the fifth day after hatching (Fig. 7E). These results suggest that *S. gigas* may switch from internal to external nutrition 4-5 days after hatching.

**Discussion**

**Sexual maturity**

Testicular structure in teleosts can be classified into two types: tubular and lobular, depending on the pattern of spermatogenesis (Billard et al., 1982). The testis of *S. gigas* are tubular, as are those of the blue-spotted mud hopper *B. pectinirostris* (Chung et al., 1991), the naked-headed goby *Fa vonigobius gymnauchen* (Lee et al., 2000), and the gluttonous goby *Chasmichthys gulosus* (Kim et al., 2004).

Teleost ovaries can be divided into three types based on the pattern of oocyte development: synchronous, group synchronous, and asynchronous (Nagahama, 1983). Oocyte development in *S. gigas* is synchronous, as determined by the GSI and histology.

The GSI was highest in May for *B. pectinirostris* (Jeong et al., 2004), but between June and mid-July for *S. gigas* (this study), demonstrating that the spawning season of *S. gigas* occurs later than that of *B. pectinirostris*. Similarly, the gonadal tissue of female *S. gigas* was in the ripe stage between June and mid-July. Our histological findings were consistent with the GSI findings.

Reports on *B. pectinirostris* spawning have been contradictory, with some studies concluding that this species spawns once a year (Zhang et al., 1989) and others suggesting that it spawns several times a year (Hoda, 1986; Chung et al., 1991; Washio et al., 1993). Washio et al. (1993) found that *B. pectinirostris* ovaries remained full of eggs even after spawning, and that fish were seen in the resting state only at the end of the spawning season, leading them to conclude that *B. pectinirostris* spawns several times during a spawning season. Kim and Jeong (2007) suggested that *B. pectinirostris* may spawn several times, based on a TL frequency distribution analysis of age-0 fish. However, our gonadal histology of *S. gigas* yielded similar results to those of Zhang et al. (1989), suggesting that *S. gigas* spawn once a year.

*Boleophthalmus pectinirostris* and *S. gigas* inhabit similar mud flat environments. Environmental factors are known to play an important role in regulating the reproductive cycles of teleosts (Lam, 1983). Successful reproduction entails the production of offspring when physical and biotic conditions are most likely to promote their survival (Miller, 1984). *Scartelaos gigas* exhibits distinct seasonality in its reproductive ability, as evidenced by marked cyclic changes in GSI and gonadal histology. It appears that these physiological changes are closely related to environmental changes in temperature, photoperiod, or availability of food.

**Early life history**

The elliptical-shaped eggs of *S. gigas* adhere to one another using many fine filaments, as is characteristic of most goby species. The function of the sperm-duct glands requires further study, but nest preparation by male gobies often entails rubbing the abdomen and urogenital papilla over a cleaned surface prior to egg deposition (Miller, 1984). The egg size of *B. pectinirostris* from the Gangjin mud flat is 1.23-1.44 mm (long axis) by 0.65-0.77 mm (short axis) (Jeong et al., 2010), similar to that of *S. gigas* (1.23-1.48 mm by 0.67-0.71 mm). The eggs were attached to the upper surface of the spawning burrow, in which only one male protected eggs. We newly found that air exists in the spawning burrow (see Fig. 7C) and may supply enough oxygen to the eggs. Mudskippers have been known to dig a Y-form burrow usually, but an L-form burrow during the spawning season (Ryu et al., 1995), consistent with our findings (Fig. 6). According to Koga and Noda (1992), *B. pectinirostris* usually dig a complex and very long burrow (its TL can reach 534 cm horizontally) but dig a simple and deep burrow vertically during hibernation.

The number of eggs produced is an indicator of species productivity. Studies have found 5,000-20,000 *B. pectinirostris* eggs per fish (Ryu et al., 1995) and 1,362-18,368 *S. gigas* eggs per fish (Park et al., 2008). It is believed that this kind of fish usually spawns fewer than 20,000 eggs. The TL of *B. pectinirostris* hatched larvae has been measured as 3.0-3.4 mm (Ryu et al., 1995) and 3.1-3.3 mm (Okiyama, 1988), similar to that of *S. gigas* larvae (2.58-3.24 mm). *Boleophthalmus pectinirostris* showed many small oil globules early in egg development, and these decreased significantly as the embryo developed (Ryu et al., 1995). *Scartelaos gigas* embryos contained two oil globules just prior to hatching, demonstrating that the number and size of oil globules change during egg development. A single oil globule was visible in *S. gigas* larvae, as seen in *B. pectinirostris* larvae (Ryu et al., 1995).

The melanophore is an important characteristic for identification of species during the larval stage (Russell, 1976; Kendall et al., 1984). *Scartelaos gigas* had one large melanophore on the ventral contour of the mid-tail from hatching until it reached 3.26 mm total length (3.13 mm notochord length). No melanophore was observed in the mid-tail region of *B. pectinirostris* at 3.1-3.3 mm (Okiyama, 1988). Accordingly, the melanophore is an important characteristic in distinguishing the two species before notochord flexion. The spawning ecology of *S. gigas* has not been studied before, and our new findings may be useful in the conservation and management of this species.

**Implications for conservation of *S. gigas***

Ryu et al. (1995) studied the distribution of *B. pectinirostris* at 17 sites on the west and south coasts of Korea, and suggested that this fish is no longer found at Boryeong and Chungnam.
due to reclamation. We found that the distribution density of *S. gigas* was relatively high in mudflats in the southwest of Korea, but few were found in southern Korea. It is thought that the habitat of *S. gigas* is highly restricted compared with that of *B. pectinirostris*, resulting from differences in habitat preference between the two species. *Scartelaos gigas* prefer high mud areas (mud > 99%) compared with *B. pectinirostris* (mud > 91%) (Kim et al., in preparation). Additionally, juveniles or young fish of *S. gigas* were not collected in the wild; however, many young *B. pectinirostris* were collected very readily in the wild (Ryu et al., 1995; Kim and Jeong, 2007), suggesting the possibility of potential population collapse of *S. gigas*. Thus, the species should be considered a vulnerable or conservation-dependent species. In particular, parental fish need to be protected during the main spawning season (June) to preserve *S. gigas* eggs.

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**References**


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