Early Life History of the Sevenband Grouper, *Epinephelus septemfasciatus* from Korea

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ABSTRACT : This study is conducted to monitor the morphological developmental features of the egg development, larvae and juvenile of *Epinephelus septemfasciatus*, the fertilized eggs were gotton using artificial insemination. Matured parents are collected from marine caged fish farms in Geomun-ri, Samsan-myeon, Yeosu-si, Jeollanamdo Korea in June 2012. The fertilized eggs were pelagic eggs containing one oil globule, and measured 0.81~0.89 mm (0.85±0.04 mm, n=50) in diameter. In regard to rearing environment, the water temperature is 21.0~23.0°C and the salinity is 32.0~33.2‰. Hatching was observed from 48 hours after fertilization, the mouth and anus of prelarvae was not opened but had egg yolk at newly hatched. 4 days after hatching, the mouth and anus of postlarvae was opened and began to eat Rotifer and was measured 2.40~2.49 mm (2.45±0.03 mm n=10) in total length. 12 days after hatching, postlarvae was measured 3.77~4.67 mm (4.27±0.33 mm) in total length, its the second pole tide of dorsal fin and the first pole tide of pelvic fin was extended longitudinally. 71 days after hatching, juvenile was measured 40.5~45.4 mm (42.6±2.04 mm) in total length. Seven bands were observed in body, and pole tides of dorsal and pelvic fins were shortened.

Key words : Egg development, Juvenile, Larvae, Sevenband grouper

INTRODUCTION

Investigations regarding early life history of fish deal with morphological and physiological characteristics of eggs as well as a vast amount of information including unique traits and developmental characteristics of species, appearing through the embryogenesis and its early growth thus, not only provide taxonomic, embryological, and ecological information but also can be utilized in various aspects such as conservation and protection of fishes resources, and farming via seed production (Song & Choi, 2000).

Sevenband grouper, *Epinephelus septemfasciatus* is classified as Perciformes and Serranidae there are 62 genus and 450 species. 12 genus and 27 species, including *E. septemfasciatus*, *E. akaara*, and *E. bruneus* are found in Korea (Kim et al., 2005). *E. septemfasciatus* generally prey on small fishes and crustacean, and inhabit in 10~20 m...
water depth with thick seaweeds; as grow, move to 100–200 m water depth around reef area.

Morphologically, their body and head are flat and like a long oval shape there are 7 dense dark brown horizontal stripes on the body while a white border line at the edge of a caudal fin (Jung, 1977; Kim et al., 2005). There are several studies have been done in Korea including investigations in regards to its phototaxis (Yang, 1987), mass mortality (Sohn et al., 1991), ovulation induction (Kim et al., 1997), nervous necrosis pathogenic virus (Sohn & Chun, 1999), behavior control (Yang, 1999), sex reversal induction (Song et al., 2005), as well as VNN developmental pattern (Kim et al., 2012). Meanwhile, there are researches have been done in other countries about the growth and patterns of crossbred game fishing (Miyailara et al., 1989), egg development and morphologic development of larvae and juvenile (Kitajima et al., 1991), feeding selectivity of larvae depending upon sizes of rotifers (Tanaka et al., 2005), artificial insemination using frozen sperm (Miyaki et al., 2006) and anomalies of larvae and juvenile (Nagano et al., 2007).

Considerable interests have been placed on E. septemfasciatus as a high value added industrial species due to its recent spiked demands and limited resources. Owing to such circumstances, meaning high demand on E. septemfasciatus, some are being imported from foreign countries thus it is warranted to put more emphasis on investigations in regard to industrialization of E. septemfasciatus farming (Lee & Go, 2003). Therefore, in the present study, we investigated increased production yield of E. septemfasciatus farming, stable seed production, elucidation of their early life history, as well as morphological development of larvae and juvenile depending upon their progress of egg development and growth maturation there by providing basic biological data.

**MATERIALS AND METHODS**

Parent fishes used in the study were obtained from a cage aquaculture in July 2012 (7×7 m; located in Geomun-ri Samsan-myeon, Yeosu-si, Jeollanamdo, Korea) fishes were aquacultured and fed with Trachurus japonicus as well as extrusion pellet for 8 years. Matured 5 fishes, both sex, with total length (TL) around 67.5 cm to 83.5 cm (74.8±5.3 cm) were chosen for the study.

Once confirmed fishes are matured, selected fishes, both sexes, were injected with human chorionic gonadotropin (HCG; 500 IU per kg body weight) and then eggs and sperms were collected through abdominal pressure. Obtained eggs and sperms were artificially fertilized using the wet method and washed twice with filtered seawater. Eggs that may exhibit high fertility rate were collected, packed using vinyl bags with oxygen, and transferred to the laboratory. The experimental conditions for egg development were maintained as follow: the temperature of water was between 21.5°C to 23.5°C and the concentration of salt was around in the range of 32.5% to 33.5%. Half of water in fish tanks (filtered seawater) was changed daily. Fifty fertile eggs were randomly selected and subjected to the measurement of egg diameter as well as the oil droplet size using a profile projector (JP V-12B; Nikon, Tokyo, Japan) up to 0.01 mm while processes of egg development were observed and photos were taken using a stereoscopic microscope (NM-40; Nikon, Tokyo, Japan) equipped with a digital camera (Coolpix 995; Nikon, Tokyo, Japan).

Morphological developments of larvae and juvenile were observed from hatching through the juvenile stage. Once hatched, fishes were accommodated in a circular fish tank made of polypropylene (100×50×80 cm) the water temperature and the concentration of salt were maintained between 22.5°C and 24.5°C and 32.0‰ to 33.2‰, respectively throughout the breeding period. The water condition for the present experiment was maintained as pond culture system for early hatching period whilst half of water in fish tanks was daily replaced after 10 days of hatching.
1. Fecundity and size of spawn

Throughout the present experiments, the fecundity of female parent of *E. septemfasciatus* was investigated and summarized in Table 1. The average TL of experimental fish was between 68.0–75.5 cm and the fecundity was found to be approximately 199,680 to 1,647,360 (average 767,520) in general the longer TL, the more of fecundity was observed. The fertile egg size was around 0.80 mm to 0.89 mm (0.82±0.02 mm) and a single oil droplet, about 0.17 mm to 0.19 mm (0.18±0.01 mm) was found.

2. Developmental processes of egg

Cleavage of artificially inseminated egg was divided into two within 1 hour and then reached to the 2 cell stage (Fig. 2A) subsequently, it was reached to 4 cell stage (Fig. 2B), 8 cell stage (Fig. 2C), 16 cell stage (Fig. 2D), and 32 cell stage (Fig. 2E) after 2, 3, 4.5, and 5.5 hours, respectively. As the cleavage was getting smaller, the egg was in the morula stage after 8 hours (Fig. 2F).

Fifteen hours later, the egg was in the blastula stage (Fig. 2G) and blastoderm was covered by yolk and reached in the early gastrula stage (Fig. 2H). Blastoderm as well as yolk were enlarging after 26 hours of insemination (Fig. 2I) followed by the formation of optical vesicle and Kuffer's vesicle after 34 hours (Fig. 2J). About 8 to 9 of myotome were formed during these developmental processes (Fig. 2K).

The Kuffer's vesicle was disappeared whilst the numbers of myotome were increased around 12 to 13 after 37 hours of hatching (Fig. 2L). Subsequently, after 43 hours of hatching, eye lenses were differentiated and then formed otic capsules at the same time, myotomes were about 17 to 18 (Fig. 2M). After 46 hours of hatching, the embryo began to move, formed the heart with heart rate around 62 to 72 per minute (Fig. 2N). Eventually, after 48 hours, the head break through the egg membrane it was becoming just before the hatching (Fig. 2O) and then initiated hatching right away (Fig. 2P).

### RESULTS
Fig. 2. Egg development stages of sevenband grouper, *Epinephelus septemfasciatus*. (A) 2 cell stage; (B) 4 cell stage; (C) 8 cell stage; (D) 16 cell stage; (E) 32 cell stage; (F) Morula stage; (G) Blastula stage; (H) Gastrula stage; (I)-(J) Formation of embryo; (K) Kuffer’s vesicles appearance; (L) 12–13 myotomes stage; (M) Formation of eyes; (N) Heart beat; (O) Hatching; (P) Hatched larvae. Scale bar = 1.0 mm.

3. Morphological development of larvae and juvenile

1) Prelarvae stage

Just after hatched, the TL of prelarvae is between 1.28 mm to 1.95 mm (1.74±0.24 mm, n=10) and the mouth and anus yet to be opened. There was one oil droplet and fins were like membrane shape and connect the dorsal center line of back head, tail, yolk and bottom of head. There were five and melanophores in the upper digestive tract, while only one or two of them, spot shaped, were shown in tail. The anus was located in approximately 62.1% behind of TL from very center (Fig. 3A). After 2 days of hatching, the TL of prelarvae was around 1.75–2.36 mm (2.05±0.21 mm) and the oil droplet was noticeably shrunken the mouth and anus were not completely opened. There was no significant difference in fins on membranes whereas the caudal fin was begun to differentiate. The yolk was almost completely absorbed and melanophore beneath a tail was shown as a single big dark spot (Fig. 3B).

2) Postlarvae stage

After 4 days of hatching, the TL of postlarvae was 2.40 mm to 2.49 mm (2.45±0.03 mm) and it developed the lower jar with further differentiated the pectoral fin with opening the mouth and anus. Melanophores were precipitated on eyes and the upper intestinal tract further it was widely and horizontally distributed over bottom of tail (Fig. 3C). The TL of postlarvae was about 2.48–2.59 mm (2.54±0.05 mm) and the oil droplet was mostly absorbed. There were two bones in the ventral fin while a fish bone was formed in the dorsal fin. Melanophores were newly formed at both ends of the ventral fin and melanophores of tail were more precipitated on the very center of the caudal peduncle (Fig. 3D).
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3). After 12 days of hatching, the TL of postlarvae was between 3.77 mm to 4.67 mm (4.27±0.33 mm) and a terminal notochord of the tail was bended 45°C, membrane like fins were differentiated and then, connected from back head, tail, and the anus. The bones were formed in the dorsal fin and the second bone was the longest there are also 2 bones developed lengthy in ventral fin.

Melanophores were appeared very dark at the end of both dorsal fin and ventral fin in addition, melanophores were widely shown throughout the abdominal cavity and bottom of tail (Fig. 4E). After 20 days of hatching, the TL of postlarvae was 4.79 mm to 5.99 mm (5.39±0.41 mm) and membrane like fin was connected through the third bone of the dorsal fin to the tail and anus. Further the anal fin started to differentiate. The numbers of dorsal fin become three and melanophores were precipitated on the back of eyes, like small spots (Fig. 4F).

After 29 days of hatching, the TL of postlarvae was between 6.01 mm and 8.90 mm (7.58±0.93 mm) and an anal fin as well as dorsal fin were beginning to differentiate and 10 to 11 melanophores were shown in abdominal cavity, like spots (Fig. 4G).

After 31 days of hatching, the TL of postlarvae was 9.72 mm to 14.7 mm (11.9±1.96 mm) and membrane fin was completely separated. Melanophores were newly found on the top of head while melanophores in the bottom tail were located in the very center of caudal peduncle. Spot like melanophores on the abdominal cavity was enlarged and shown like a big dot (Fig. 4H).

After 38 days of hatching, the TL of postlarvae was 12.2 mm to 17.1 mm (14.4±1.85 mm) and second bone of the dorsal fin as well as first bone of the ventral fin was further developed. Melanophores present in the very center of the caudal peduncle were widely precipitated like spots (Fig. 5I). After 44 days of hatching, the TL of postlarvae was around 15.6 mm to 18.9 mm (17.3±1.40 mm) and by and large, melanophores were found in bottom of eyes, upper part of operculum, and head through caudal peduncle, further there were more of melanophores, precipitated like dots on back side, an anal fin bone, as well as its bottom part (Fig. 5J). After 52 days of hatching, the TL of postlarvae was between 18.3 mm to 26.2 mm (21.3±2.17 mm) and second bone of the dorsal fin and first bone of the ventral fin gradually got shorter and precipitated melanophores found in the whole body got darker in color, eventually 7 horizontal stripes were shown (Fig. 5K).

3) Juvenile stage

After 61 days of hatching, the TL of juvenile is around 30.7 mm to 34.5 mm (32.9±1.38 mm) and second bone of the dorsal fin and first bone of ventral fin were almost got shorten. Melanophores found in the head and an operculum got darker.

They were also widely precipitated on back side, abdomen,
Fig. 5. Development of postlarvae and juvenile seven-band grouper, *Epinephelus septemfasciatus*. (I) 14.4 mm in TL postlarvae, 38 days after hatching; (J) 17.3 mm in TL postlarvae, 44 days after hatching; (K) 21.3 mm in TL juvenile, 52 days after hatching; (L) 32.9 mm in TL juvenile, 61 days after hatching; (M) 42.6 mm in TL juvenile, 71 days after hatching. Scale bars = 1.0 mm.

Throughout the breeding period of *E. septemfasciatus* larvae and juvenile, relative growth of the part length was shown in Fig. 6 and 7 respectively and calculated as follow.

\[
\begin{align*}
\text{TL} &= 1.2820 \times \text{BL} - 0.4984 \quad R^2 = 0.9985 \\
\text{PL} &= 0.7046 \times \text{BL} - 0.6989 \quad R^2 = 0.9960 \\
\text{HL} &= 0.4160 \times \text{BL} - 0.3284 \quad R^2 = 0.9961 \\
\text{ED} &= 0.1441 \times \text{BL} - 0.0329 \quad R^2 = 0.9427 \\
\text{BD} &= 0.4666 \times \text{BL} - 0.6429 \quad R^2 = 0.9912 \\
\text{UJL} &= 0.1583 \times \text{BL} + 0.1880 \quad R^2 = 0.8702
\end{align*}
\]

The TL respect to the BL was slowly growing from 1.90 mm to 6.56 mm, subsequently it was growing fast in the juvenile stage when the TL was between 21.9 mm and 38.4 mm, when all fins and muscles were developing. The preanal length respect to BL was growing slowly between 0.58 mm and 3.15 mm in the early larvae period when start to develop the intestinal tract. Afterward, once developed the intestinal tract, the anus, located in front part of the body moved to the very centre of the body and showed fast growth from 11.5 mm through 22.0 mm.

The HL respect to BL was also growing slowly from 0.58 mm to 1.93 mm in the early larvae period while it grew fast in between 6.84 mm and 12.3 mm. The ED, respect to the BL grew up slowly between 0.15 mm and 0.24 mm; this was the period when melanophores were began to precipitate in eyes and all yolk was absorbed. In contrast, there was slow growth from 2.65 mm to 4.16 mm after melanophores were completely precipitated. The BD respect to BL was growing slowly in between 0.55 mm (right after the hatching) and 2.01 mm when yolk was absorbed in the postlarvae stage, it was growing fast between 6.62 mm to 14.7 mm when the metamorphosis was proceeding. The UJL respect to the BL was growing slowly in between 0.30 mm and 0.67 mm this might be because early larvae is not very active and the amount of food intake is not significant. After this, there was an
Fig. 6. Relative growth of total length, preanal length, head length and eye diameter against body length in larvae and juvenile sevenband grouper, *Epinephelus septemfasciatus*.

Fig. 7. Relative growth of body depth and upper jaw length in larvae and juvenile sevenband grouper, *Epinephelus septemfasciatus*.

increase in food intake between 2.83 mm and 4.81 mm and a fast growth as both lower and upper jaws were developing.

**DISCUSSION**

Fertile eggs of *E. septemfasciatus* are colorless, transparent, and spherical, they are characterized by a separated pelagic egg with single oil droplet (Liao & Leano, 2008).

The average diameter of fertile eggs is about 0.85 mm in the study which is in good agreement with previous reports elsewhere Lee & Go (2003) and Kitajima et al. (1991). Compared to other Serranidae, it was slightly bigger than those of *E. akaara* 0.71–0.82 mm (Song, 1998) and Humpback grouper, *Cromileptes altivelis* 0.83 mm (Bulanin et al., 2005) while relatively smaller than *E. bruneus* 0.90 mm (Lee & Go, 2003) and Nassau grouper, *E. striatus* 0.92 mm (Allyn et al., 1992). It has been widely known that water temperature impacts on not only the development of fish egg but the growth and survival rate of larvae and juvenile in the seed production processes.

In general, higher water temperature facilitates the development of eggs while low temperature delays it. Therefore, each fish represents their optimal range of water temperature given their specific life style, ecological characteristics for normal development (Hokanson et al., 1973; Gunnes, 1979; Herzig & Winkler, 1986; Rana, 1990).

When it comes to time consuming for hatching, in case of *E. septemfasciatus*, it took 48 hours when the water temperature was around 21.5–23.5°C in the study. According to Lee & Go (2003), it took 46 hours and 35 hours at 22.0°C and 25.0°C of water temperature, respectively. Further, Kitajima et al. (1991) have shown that it takes approximately 30–35 hours for *E. septemfasciatus* to hatch at 21.2–23.2°C of water temperature. For *E. akaara*, it took 25.5 hours at 25.0°C of water temperature (Song, 1998) while *E. bruneus* needs 32 hours at 25.0°C of water temperature to hatch (Lee & Go, 2003). Humpback grouper required 20 hours and 10 minutes at 27.0–28.0°C of water temperature (Bulanin et al., 2005) while Nassau grouper needed 27–29 hours at 23.0–30.0°C, respectively. Taken together, it seems generally true that most Serranidae fishes tend to require shorter time to hatch with higher water temperature, indicating close association between water temperature and time required for hatching eggs.

These results seem to be general in the development of eggs of many species such as *Girella punctata* and *G. melanichthys* (Oh et al., 2010), *Paralichthys olivaceus*...
When compare the TL of larvae right after hatching, the average of TL of *E. septemfasciatus* was 1.74 mm which is very closed to other studies elsewhere Lee & Go (2003) and Kitajima et al. (1991). This was slighter longer than the average TL of *E. akaara* 1.65 mm (Song, 1998) as well as *E. fasciatus* 1.30~1.40 mm (Kawabe et al., 2009) while shorter than those of Humpback grouper 1.86 mm (Bulanin et al., 2005), Nassau grouper 1.70~1.80 mm (Allyn et al., 1992) as well as *E. bruneus* 2.02 mm.

Serranidae fishes have been known to go through the metamorphosis processes that represent significant transformation in the postlarvae stage. In the study, we considered to be started as the second spinous ray of the dorsal fin and the first spinous ray of the ventral fin got elongated. When it comes to the timing, *E. septemfasciatus* began metamorphosis after 8 days of hatching and the TL was between 2.48~2.59 mm. In other studies, for example, Lee & Go (2003) reported that it was started after 9 days of hatching and TL was around 2.94 mm. Further, Kitajima et al. (1991) reported that it initiated its metamorphosis after 13 days of hatching and the average TL was 3.45 mm. Such morphological changes are shown in Pleuronectiformes fishes such as *P. olivaceous* (Han & Kim, 1997), *Pleuronectes herzensteini* (Han & Kim, 1999), and *Platichthys stellatus* (Byun et al., 2007). These Pleuronectiformes fishes used to have their eyes separated in prelarvae stage but represent morphological changes there by moving one eye to the other side and directing their body toward bottom. *E. septemfasciatus* only represent their partial morphological changes in spinous ray of the dorsal and ventral fins (known as late onset metamorphosis), while Pleuronectiformes fishes generally go through the significant metamorphosis. Even though it is somewhat difficult to determine the exact

| Species               | Egg size (mm) | Hour of hatched (water temp.) | Total length of newly hatched larvae (mm) | Days of metamorphosis total length (mm) | Juvenile (mm) | References               |
|-----------------------|---------------|-------------------------------|------------------------------------------|----------------------------------------|---------------|--------------------------|
| *Epinephelus septemfasciatus* | 0.85          | 48 h (21~23℃)                | 1.74                                     | 8 days (2.54)                           | 61 days (32.9)| Present study            |
|                       | 0.82          | 46 h / 35 h (22℃ / 25℃)      | 1.75                                     | 9 days (2.94)                           | -             | Lee & Go, 2003           |
|                       | 0.82          | 30~35 h (21.2~23.2℃)         | 1.85                                     | 13 days (3.45)                          | 60 days (30.0)| Kitajima et al., 1991   |
| *Epinephelus akaara*  | 0.71~0.82     | 25 h 30 min (25℃)            | 1.65                                     | -                                      | -             | Song, 1988               |
| *Epinephelus striatus* | 0.92          | 27~29 h (23~30℃)             | 1.70~1.80                                | -                                      | 40 days (13.5)| Allyn et al., 1992      |
| *Epinephelus bruneus* | 0.90          | 32 h (25℃)                   | 2.02                                     | -                                      | 50 days (10.5)| Lee & Go, 2003           |
| *Epinephelus fasciatus* | -             | -                            | 1.30~1.40                                | -                                      | 30 days (12.4)| Kawabe et al., 2009     |
| *Cromileptes altivelis* | 0.83          | 20 h 10 min (27~28℃)         | 1.86                                     | -                                      | -             | Bulanin et al., 2005    |
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size and timing of metamorphosis for larvae, there might be interspecies differences depending upon their growth, warranting further investigation regarding larval breeding as well as their growth.

When comparing the days to migrate to the juvenile stage, *E. septemfasciatus* took about 61 days after hatching the average TL was 32.9 mm. On the other hand, Kitajima et al. (1991) reported that it took about 60 days and their average TL was also similar average 30.0 mm. In case of *E. bruneus*, it took about 50 days for an average and the TL was around 10.5 mm (Lee & Go, 2003). According to Kawabe et al. (2009) it took 30 days for *E. fasciatus* to proceed the juvenile stage and their average BL was 12.4 mm (Kawabe et al., 2009) while Nassau grouper (Allyn et al., 1992) required 40 days for average and BL was 13.5 mm (Table 2).

Taken altogether, in the present study, we investigated the egg developmental processes in seed production through the investigation of the early life history of *E. septemfasciatus* and the required time as well as morphological development stages of larvae and juvenile throughout their growth. There are still many difficulties in regard to seed production of *E. septemfasciatus* and it is warranted to investigate further in particular about hormone induced spawning, production of improved quality of eggs, organized management of parent fishes, elucidation of maturation timing, and selection of hormone application.

**ACKNOWLEDGEMENT**

This research was supported by grants from the National Fisheries Research and Development Institute (NFRDI) in Korea (RP-2014-AQ019).

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