Onset and development of aggressive behavior in the early life stages of the seven-band grouper *Epinephelus septemfasciatus*

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Abstract

Onset and development of aggressive behavior were observed in the early life stages of seven-band grouper *Epinephelus septemfasciatus*. Fish culture was divided into two terms: the first term, from hatch until 21 days after hatching (DAH); and the second term, from 21 DAH until settlement (65 DAH). During the second term the effect of different aeration rate on survival was investigated. Survival during the first term was 14.1±7.1%. In the second term, survival in control tanks (aeration rate 200 mL/min) was 14.7±10.2% and 18.8±7.8% in the increasing aeration tanks (aeration rate 200-800 mL/min). Behavioral observations were conducted at about 8-days intervals and aggressive behavior was quantified by the frequency of chase behavior. Aggressive behavior was first observed on 52 DAH when pigment appeared on the dorsal area of the metamorphosing larvae (standard length 16.6±6.0 mm). Aggressive behavior significantly increased from 59 DAH coinciding with the beginning of settlement.

1 Introduction

Behavioral development is the key to understand the life mode of fish larvae and juveniles in relation to morphological development and organogenesis (Fukuhara, 1992). Many fish species develop social responses in their early life stages (Noakes and Godin, 1988). Social behavior includes all behaviors directly related to actual or potential encounters between individuals within a species (Noakes, 1978), such as aggressive behavior and cannibalism (Sakakura and Tsukamoto, 1999). Aggressive behavior, including cannibalistic behavior, has a significant impact on the early life history of fishes not only in the rearing conditions but also in the wild (Smith and Reay, 1991). Investigation of development of social behavior is of practical importance to improve the quality of reared fish for stock enhancement (Olla et al., 1994; Svâsand, 1993; Sakakura, 2006) and aquaculture.

Groupers (subfamily Epinephelinae) include many commercially important species and have become target species for stock enhancement and aquaculture in Japan (Fukuhara, 1989). However, high mortalities due to aggressive behavior and/or cannibalism from the end of the metamorphosis to the juvenile stage had been reported in many grouper species, such as red-spotted grouper *Epinephelus akaara* (Morizane et al., 1984, Kayano and Oda, 1986, Narita et al., 1986), orange-spotted grouper *E. coioides* (Duray et al., 1997, Bombeo-Tuburan et al., 2001, Takeshita and Soyano, 2008), brown-marbled grouper *E. fuscoguttatus* (Lim, 1993), giant grouper *E. lanceolatus* (Hseu et al., 2004, 2007), camouflage grouper *E. microdon* (Okinawa PFES, 1984), long-tooth grouper *E. moara* (Nakagawa, 1988), greasy grouper *E. tauvina* (Lim, 1993) and potato grouper *E. tukula* (Yeh et al., 2003). Since it is possible to reduce cannibalism in finfish culture with size-grading techniques (Liao et al., 2001), it is

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crucial to investigate the onset of aggressive behavior to reduce mortality by cannibalism for increasing survival in the rearing of these species.

Seven-band grouper *E. septemfasciatus* is a candidate species of aquaculture in Japan. However, mortality due to cannibalism is a bottleneck for improving seedling production (Kitajima et al., 1991). Early development of this species had been well described by Kitajima et al. (1991) and there are studies on the growth and survival of larval stages in relation to the effect of food selectivity (Tanaka et al., 2005), water flow field/aeration (Shiotani et al., 2003, 2005, Sakakura et al., 2006, 2007) and tank proportion (Ruttanapornvareesakul et al., 2007). However, there is no quantitative report on the development of aggressive behavior of the seven-band grouper. The objective of this study is to investigate the onset and development of aggressive behavior of seven-band grouper from hatch until the juvenile stage.

2 Material and Methods

2.1 Egg collection and general rearing

A female broodstock (5.2 kg body weight), which was caught in the wild in 2003, was reared in a floating net cage (5x5x5 m) at the Nagasaki Prefectural Fisheries Experimental Station, Nagasaki, Japan. It was subjected to the hormonal treatment described by Ni Lar Shein et al. (2004). Fertilized eggs were obtained by artificial insemination using cryo-preserved sperm (Miyaki et al., 2005). Floating eggs were transferred into 1,000-L circular black rearing tanks at a density of 10 eggs/L and hatched approximately 24 hours after transfer. To calculate hatching rate 100 eggs were transferred into a 1-L beaker, incubated at $20^{o}C$ and hatched larvae were counted every 24 hours until all eggs hatched or died. The rearing of seven-band grouper was divided

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in two terms: from hatch until 21 days after hatching (DAH), and from 21 DAH to 65 DAH. Survival was calculated at the end of the first and second terms.

Rotifers *Brachionus plicatilis* sp. complex (Hagiwara et al., 2007) were cultured in 1,000 L transparent tanks with HUFA enriched *Chlorella vulgaris* (Super *Chlorella* V-12; Chlorella Industry Co. Ltd., Fukuoka, Japan) and newly hatched *Artemia* nauplii enriched with Super Capsule Powder A-1 (Chlorella Industry Co. Ltd., Fukuoka, Japan).

2.2 Rearing conditions in the first term (0-21 DAH)

Larval rearing during the first term was designed according to the previous trials for the seven band grouper (Tanaka et al., 2005). Two black rearing tanks, each containing 1,000 L of seawater, were kept in a water bath at 25.4±0.9 ℃ with an aeration of 200 mL/min and natural light condition ranging 97-673 Lux during daytime on the water surface. Surface film was formed once a day by the addition of oil at 0.2 mL/m^2 water surface (Riken Feed Oil Omega; Riken Vitamin, Tokyo, Japan) from 3 DAH until the end of the experiment to prevent surface tension-related death of larvae (Yamaoka et al., 2000, Tsuchihashi et al. 2003). Sand filtered seawater with UV disinfection was supplied at water exchange rate of 100%/day.

Larvae were fed once a day with SS-type rotifers from mouth opening (3 DAH, standard length, SL, mean±SD, 2.3±0.1 mm) until 13 DAH (SL 2.3±0.1 mm) at a density of 10 ind./mL, and L-type rotifers from 14 DAH until 21 DAH at a density of 10 ind./mL, respectively. Super *Chlorella* V-12 was added from mouth opening at a density of 50x10^5 cells/mL once daily.

2.3 Rearing conditions in the second term (21-65 DAH)

In the second term, fish survival was compared when reared at two different aeration rates, constant 200 mL/min aeration and increasing aeration rate (200 mL/min between 21-26 DAH; 400 mL/min between 27-44 DAH; 600 mL/min between 45 and 53 DAH; and 800 mL/min from 53 DAH until the end of the experiment). Two 1,000-L black rearing tanks were prepared for each aeration treatment and were kept in a water bath at 25-26 $^{\circ}$C. Light condition was natural ranging 37-706 Lux during daytime on the water surface and sand filtered seawater with UV disinfection was supplied at water exchange rate of 100%/day. One thousand larvae from the first term were transferred into each tank.

Larvae were fed once a day with L-type rotifers from the beginning of the trial until 45 DAH at a density of 10 ind./mL, and *Artemia* from 30 DAH until the end of the experiment at a density of 0.03-2.0 ind./mL twice daily. Other rearing conditions were kept same as those in the first term rearing trial.

2.4 Sampling

Thirty fish were randomly taken from the control tanks at 0, 5, 13, 15 and 20 DAH during the first term and 25, 30, 40, 50, 55 and 66 DAH during the second term. Fish were anesthetized with MS222 (Tricaine; Sigma Chemical Co., St Louis, MO, USA) and preserved in 5% formalin solution. To measure SL, a digital microscope (VH-6300, Keyence Corp., Osaka, Japan) or a digital caliper (CD-15CP, Mitutoyo Corp., Nakagawa, Japan) was used. Scientific drawings of the sample were made for each developmental stage using a dissecting microscope with camera lucida (Olympus SZX-12, Olympus Optical Corp., Tokyo, Japan) following Kitajima et al. (1991).

We realized that the former definition of developmental stages (Kitajima et al., 1991) is not appropriate, because it defined individuals that still have some larval characteristics, such as elongated spines and no coloration (Kendall et al., 1984, Trijuno et al., 2002, Kato et al., 2004, Hussain and Higuchi, 1980), as juveniles. Therefore we examined the development of digestive tract together with the morphological development during the early life stages of seven-band grouper and proposed a new developmental stage (Fig. 1).

2.5 Behavioral observations

Observations were performed in order to examine the behavioral development of the seven-band grouper in 5 developmental stages: hatch (0 DAH), development of dorsal and pelvic spines (13 DAH), flexion (24 DAH), metamorphosing larvae (dorsal and pelvic spines longest respect to the body length, 38 DAH), late metamorphosing stages with pigmentation on the dorsal region (beginning of settlement, 52 DAH) and juvenile with mature color pattern (63 DAH). Behavioral indexes were defined as follows: swimming speed; relative swimming speed (swimming speed/SL); and chase (an aggressive fish bursts towards and follows another fish for a short time).

Video recordings to measure swimming speed and relative swimming speed were performed using observation containers in an enclosed chamber at 1,000 lx. Five fish were gently transferred from the rearing tank into an observation container with seawater from the rearing tank at 9:00. Three different containers were used according to fish size, 50 mL (5 cm (L), 4 cm (W) and 3 cm (H)), 350 mL (10.5 cm (L), 8 cm (W) and 6 cm (H)) or 5 L (24 cm radius, 12 cm height). Video recordings of fish behavior were made from above for 60 minutes. After observation, fish were anesthetized with

MS222 and fixed in 5% formalin solutions. Swimming speed of each fish was calculated by the computer program Larvae version 0.9, which was developed by the cooperation of Aquaculture Biology Laboratory, Nagasaki University and Dr. Nobuyoshi Taguchi at Technology Center of Nagasaki Prefecture, Nagasaki, Japan. SL, total length (TL), dorsal spine length and pelvic spine length were measured and developmental stage was determined.

Aggressive behavior was directly observed in observation containers. According to fish size, either 1 L crystal beaker (11 cm diameter) or 5 L white plastic tank (24 cm diameter) were used under natural light condition. Two to four replicates were performed in every sampling day. Five fish were introduced into an observation tank and behavior was observed for 10 minutes every 2 hours from 9:00 to 17:00. At the end of the observation, fish were anesthetized with MS222 and fixed in 5% formalin solution. SL, TL, dorsal spine length and pelvic spine length were measured and the development stage was determined. Frequencies of chase behavior were pooled by hour and age and mean value (per minute per fish) was obtained for each time and age group, respectively. Twenty minutes observations were made daily for each rearing tank together with the observation containers.

Using the data of SL of fish from the behavioral observations, specific growth rate (SGR) and coefficient of variation (CV) were calculated for each age group. CV was calculated as:

$$CV = SD/SL$$

where CV is the coefficient of variation, SD is standard deviation and SL is average standard length.

SGR was calculated as:

$SGR=SL_2-SL_1/t$

where SL_1 and SL_2 are the SL of the fish at the beginning and at the end of a period of t days.

2.6 Statistical analysis

To determine whether there was a difference in growth or behavioral parameters among developmental stages, one-way ANOVA was performed ($p<0.05$). In case significant difference was detected, Tukey-Kramer test was used to assess differences among hours ($p<0.05$), and if no significant differences were detected, data were pooled and mean values were calculated for each sampling day. Tukey-Kramer test was also used to assess differences among sampling days ($p<0.05$). To compare survival and SL in the second term between control and increasing aeration tanks χ^2-test and *t*-test were performed, respectively ($p<0.05$).

3. Results

3.1 Growth, development and survival

Fertilization rate and hatching rate were 95% and 100% respectively. The SL at hatching was 1.6±0.1 mm (n=30, Fig. 1). At mouth opening yolk sac and oil globule were almost absorbed (4 DAH, SL 2.3±0.1 mm, Figs. 1 and 2). Survival during the first term (from hatch until 21 DAH) was 14.1±7.1% and the SL of the larvae was 3.7±0.6 mm. Survival in the increasing aeration tanks was 18.8±7.8% and 14.7±10.2% in the control tanks during the second term. There was no significant difference in SL of fish between the increasing aeration tank (SL 25.9±1.9 mm) and the control (SL 25.3±1.9

mm) at the end of the experiment (65 DAH). The relationship between SL and TL was described by the formula: TL = 1.0072 SL$^{1.0785}$ (R = 0.9986, n=265).

The seven-band grouper developmental stages were classified as:

A: Yolk sac, immediately after hatch (SL 1.2 mm, Fig. 2). Yolk and oil globule clearly visible. No pigmentation is present in the eye. Anus located in the posteroventral area but not open.

B: Mouth opening, 5 DAH (SL 2.4 mm). Yolk and oil globule completely absorbed. Mouth and anus opened and eyes fully pigmented. Anus migrates to a centroventral position. The primitive digestive tract which enables larva to feed on exogenous food is established. Intestine and rectum visible but undeveloped. Pelvic fin develops. Melanophores form a cap on the dorsum of the gut.

C: Pelagic larvae, 13 DAH (SL 3.2 mm). Digestive tract rotates and develops. The second dorsal spine and the pelvic spines primordium develop. Nostrils begins to be distinguished.

D: Pre-flexion larvae, 15 DAH (SL 3.5 mm). Digestive tract stops rotating and increases in capacity. The second dorsal spine and the pelvic spines elongate and develop melanophores in their distal end.

E: Flexion larvae, 20 DAH (SL 6.0 mm). Skull clearly visible. Pre-opercle, opercle and inter-opercle developed. First and third dorsal fin ray appear, and the second dorsal spine and the pelvic spines elongate further with a serrated border. Notochord flexion begins and caudal fin develops. Stomach and intestine increase in capacity.

F: Post-flexion larvae, 25 DAH (SL 6.9 mm). No change is visible in the development of the digestive track, but increases in capacity. Hard fin ray count

increases without reaching the adult fin ray complement. Soft rays develop in dorsal and anal fin. Notochord fully flexed and caudal fin nearly completed. A depression is visible in the nostrils.

G: Metamorphosing larvae, 30 DAH (SL 8.1 mm). No development of the digestive track, but bigger capacity. The second dorsal spine and the pelvic spines reach its maximum length respect to the body. All fins fully developed with a complete hard and soft fin ray count. Melanophores develop in the head.

H: Metamorphosing larvae, 40 DAH (SL 9.7 mm). Operculum well developed. Pigmentation more extensive in the head. Appearance of melanophores around the opercle. No development of the digestive tract, but bigger capacity.

I: Metamorphosing larvae, 50 DAH (SL 12.2 mm). Stomach and intestine begin to differentiate, both increase in capacity. The second dorsal spine and the pelvic spines still long compare to the body, but begin to decrease its relative length. Body shape similar to adult. Pigmentation appears in the base of the dorsal fin.

J: Metamorphosing larvae, 55 DAH (SL 15.5 mm). Pyloric caeca differentiates. Skull well developed. The second dorsal spine and the pelvic spines continue to decrease in length.

K: Juvenile, 66 DAH (SL 20.5 mm). Pyloric caeca well differentiated. Proportion and coloration identical to adults. Lateral line system clearly visible from the opercle to the base of the caudal fin. Body covered in melanophores and 7 bands clearly visible. Nostrils bridge does not develop completely.

The second dorsal spine and pelvic spines appeared from 14 DAH (SL 2.5±0.2 mm) and reached half the length of the body by 20 DAH. Metamorphosing larvae with completion of fin rays with maximal length of spines to the SL (over 100% for second

dorsal spine and around 80% for pelvic spines, Fig. 3) were first observed from 31 DAH (SL 5.5±0.6 mm). From 51 DAH (SL 16.6±6.0 mm), metamorphosing larvae with pigmentation on the dorsal region were firstly observed and their second dorsal spine and pelvic spines decreasing in length compared to the SL (around 40% for second dorsal spine and around 30% for pelvic spines). CV of SL increased steadily from hatch (5%, Fig. 1) until the beginning of metamorphosis (40 DAH, 21.9%) and became stable thereafter. SGR decreased rapidly from hatch (0.39 day^{-1}, Fig. 1) until the mouth opening stage (0.10 day^{-1}) and stayed stable until 36 DAH. Afterwards, SGR became very variable with a slight trend to increase towards the end of the metamorphosis stages.

3.2 Behavioral development

Swimming speed did not increase significantly from hatch (13.5±6.0 mm/sec) until 40 DAH (metamorphosing larvae, 24.4±15.0 mm/sec, ANOVA, df=9, *F*=97.108, *p*<0.0001, Fig. 4). Swimming speed increased significantly from the appearance of pigmentation on the dorsal area during the late metamorphosing stages (52 DAH, 99.1±38.6 mm/sec) and showed a peak at 56 DAH (165.5±89.4 mm/sec).

Relative swimming speed decreased steeply from hatch (7.9±3.6 mm/SL/sec, ANOVA, df=9, *F*=35.122, *p*<0.0001, Fig. 4) until the metamorphosing larvae stages (35 DAH, 3.5±5.1 mm/SL/sec). From the end of the metamorphosing larvae stage the relative swimming speed increased until its peak at 56 DAH (9.0±4.9 mm/SL/sec).

As there was no effect of hour of the day on the expression of aggressive behavior data were pooled and mean values were calculated for each sampling day. No

aggressive behavior was observed from hatch until the late metamorphosing larval stages at the appearance of pigmentation in the dorsal area (51 DAH, Fig. 5). Afterwards, aggressive behavior increased significantly from the appearance of pigmentation until the end of the experiment (ANOVA, df=10, F=64.916, p<0.0001).

Settlement was first observed in the rearing tank towards the end of the experiment in metamorphosing larvae with an almost mature color pattern (towards the end of stage J, 59 DAH).

4 Discussion

Larval growth and development in our rearing experiment were comparable to the former study of this species (Kitajima et al., 1991), even though we had a very variable SGR during the last days of our experiment. On the other hand, survival showed higher trend for the metamorphosing larvae and juveniles in the increasing aeration tanks than in other study (1.3% at 25°C using feed oil and natural light condition, Tsuchihashi et al., 2003). These results support the data and hypothesis by Sakakura et al. (2006) that an optimized aeration throughout the development of seven-band grouper is necessary for improvement of survival of larviculture of this species.

Grouper species change their habitat from a relatively calm environment with slow currents at open-ocean during the larval and early metamorphosing stages to an environment with stronger currents in shallow rocky shoals during the late metamorphosing and juvenile stages (La Mesa et al., 2002). Hence, the higher survival in the different aeration tanks suggests that the physical rearing environment of water flow was optimized for the fish. We hypothesize that the change in the aeration according to the fish size will increase the survival of the fishes, not only by increasing

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the encounter rate of live feed to the fish without producing excessive stress on the seedlings (rotifer density was kept constant) but also by optimizing the rearing environment to the fish swimming capabilities by reflecting the natural history of this species.

Fast swimming speed and relative swimming speed during the yolk sac and mouth opening stages (Fig. 4) can be attributed to a predator avoidance tactic displayed as a dispersion of the larvae after hatching (Fuiman and Magurran 1994). Although swimming speed did not change until the late metamorphosing larval stages, relative swimming speed was very low during the beginning of metamorphosing larval stages. This slow relative swimming speed stages coincides with the longest dorsal and pectoral spine lengths, probably as a result of the lag produced by the dorsal and pelvic spines. Well developed spines in metamorphosing grouper larvae may constitute a trade off between swimming ability and larval protection against predators (Fuiman and Magurran, 1994) during the pelagic stages of the development before settlement. Increase in swimming speed and relative swimming speed were observed from the beginning of settlement. The high swimming speed on this species during the late metamorphosing larvae and juvenile stages may indicate migratory movements of the fish from spawning grounds to nursery areas. Also, a higher swimming speed could be beneficial for territorialism, as an increase in swimming speed may be useful for the fish in the search and protection of a suitable habitat and in feed search/predator avoidance. We propose that seven-bend grouper juveniles use a strategy of fast growth and development as well as a fast swimming speed in order to minimize the risk of predation from the beginning of the metamorphosis.

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Aggressive behavior appeared after the relative length of the second dorsal and pelvic spines began to decrease (40% and 25% of the SL or less, respectively), indicating that certain development during the metamorphosis must be achieved for the onset of aggressive behavior. These spines develop during the pre-flexion stage larvae as protection structures from predation (Fuiman and Magurran, 1994). Similarly to the data reported by Kitajima et al. (1991), we observed that the maximum relative length of the spines coincided with the beginning of metamorphosis (about 80% for the dorsal fin and 46% for the pectoral fin, Fig. 3). Behaviorally, the onset of the aggressive behavior coincided with the beginning of the settlement in seven-band grouper, presumably as a beginning of territorialism. Seven-band grouper developed aggressive behavior relatively late in terms of age (51 DAH), compared to other grouper species such as *E. moara* (30 DAH, Nakagawa, 1988, 35 DAH, Narita et al., 1986) or *E. microdon* (30 DAH, Okinawa PFES, 1984). However, in terms of total length seven-band grouper aggressive behavior onsets at a comparable size (TL 20.9±8.0 mm) to other grouper species such as *E. moara* (TL 18 mm, Narita et al., 1986), *E lanceolatus* (TL 19 mm, Hseu et al., 2004) or *E coioides* (TL 15-16 mm, Narisawa et al., 1997, Hseu et al., 2003). Although we did not check aggressive behavior during nighttime, we speculate that aggressive behavior will mainly occur during daytime, since groupers are visual feeders (Yoseda et al., 2008).

When compared with other settling fish, such as Japanese flounder (Sakakura and Tsukamoto, 2002), aggressive behavior of seven-band grouper onsets at a comparable developmental stage, around the end of metamorphosis. In other species, such as yellowtail (Sakakura and Tsukamoto, 1999), aggressive behavior also onsets from the beginning of the juvenile stage. Settlement of seven-band grouper was seen

from a comparable developmental stage to Japanese flounder (Sakakura and Tsukamoto, 2002), towards the end of metamorphosis (appearance of bands in the dorsal area).

Size variations in SL increased slowly in the development of seven-band grouper until the metamorphosing stages and CV remained relatively stable after the onset of aggressive behavior (CV<25%). Aggressive behavior related growth dispensation has also been observed on juvenile cichlids (*Tilapia zillii*, Koebele, 1985), where not only direct interactions of dominants but also visual cues affected negatively the growth of subordinates. This effect will be reinforced by the high growth rates generally showed by dominants due to an even greater competitive ability and therefore strengthen the position of the dominant fish in the hierarchy (Sloman and Armstrong, 2002). Although the similar experiments in yellowtail *Seriola quinqueradiata* showed less variation in SL (CV<15%, Sakakura and Tsukamoto, 1996), yellowtails showed the same ontogenetic changes in CV. In the wild, schools of juvenile yellowtails consisting of similar ages aggregate to drifting seaweed in current rips (Sakakura and Tsukamoto, 1997). It is assumed that aggressive behavior and social rank in schools of yellowtail function to make the body size of school members uniform both in the wild and artificial rearing conditions in order to minimize individual predation risk by predator confusion (Sakakura and Tsukamoto, 1999). However, this is not the case in seven-band grouper, which does not show schooling behavior. Seven-band grouper late metamorphosing larvae and juveniles settle at inshore regions and they inhabit shallow rocky shores. There is not a shift toward deeper waters with increasing size in groupers, but rather an enlargement of their bathymetric range, at least for undisturbed populations (La Mesa et al., 2002). Observations from coral reef habitats indicated that settled juvenile groupers are cryptic fishes, closely associated with the bottom, and not

straying far from crevices (Smith, 1961). Habitat dependency of tropical groupers was probably more closely related to the need for shelter than for food (Parrish, 1987). Thus we assume that aggressive behavior in the early seven-band settling grouper may have a function for shelter/resources protection.

5 Acknowledgements

6 References

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Figure captions

Fig. 1. Growth of the seven-band grouper. A: Solid dots indicate standard length and bars indicate standard deviations (n=4-44). Significant differences among ages are indicated by alphabets (Tukey-Kramer test, $p<0.05$, a<b<c<d<e<f<g). B: Solid dots indicate coefficient variation of standard length and clear dots Specific Growth Rate (SGR) for each age group.

Fig. 2. Morphological development and development of digestive tract of the seven-band grouper. The developmental stages of fish were defined as: A, Yolk sac (0 DAH, SL 1.2 mm); B, Mouth opening (5 DAH, SL 2.4 mm); C, Pelagic larvae (13 DAH, SL 3.2 mm); D, Pre-flexion (15 DAH, SL 3.5 mm); E, Flexion (20 DAH, SL 6.0 mm); F, Post-flexion (25 DAH, SL 6.9 mm); G, Metamorphosing larvae (30 DAH , SL 8.1 mm); H, Metamorphosing larvae (40 DAH , SL 9.7 mm); I, Metamorphosing larvae (50 DAH, SL 12.2 mm); J, Metamorphosing larvae (55 DAH , SL 15.5 mm); K, Juvenile (66 DAH , SL 20.5 mm). The scale bar of each scientific drawing represents either 0.5 mm (drawings A, B, C, D and E) or 1 mm (drawings F, G, H, I, J and K).

Fig. 3. Changes in spine length as a percentage of the body length during early life stages of seven-band grouper (A: Second dorsal spine. B: pelvic spines). Open dots indicate aggressive fish and solid dots indicate subordinates, respectively.

Fig. 4. Changes in swimming behavior of seven band grouper (for hatch n=10, the other n=5). A: Swimming speed. B: Relative swimming speed. Bars indicate standard deviations. Significant differences are indicated by alphabets (Tukey-Kramer test, $p<0.05$, a>b>c>d>e>f).
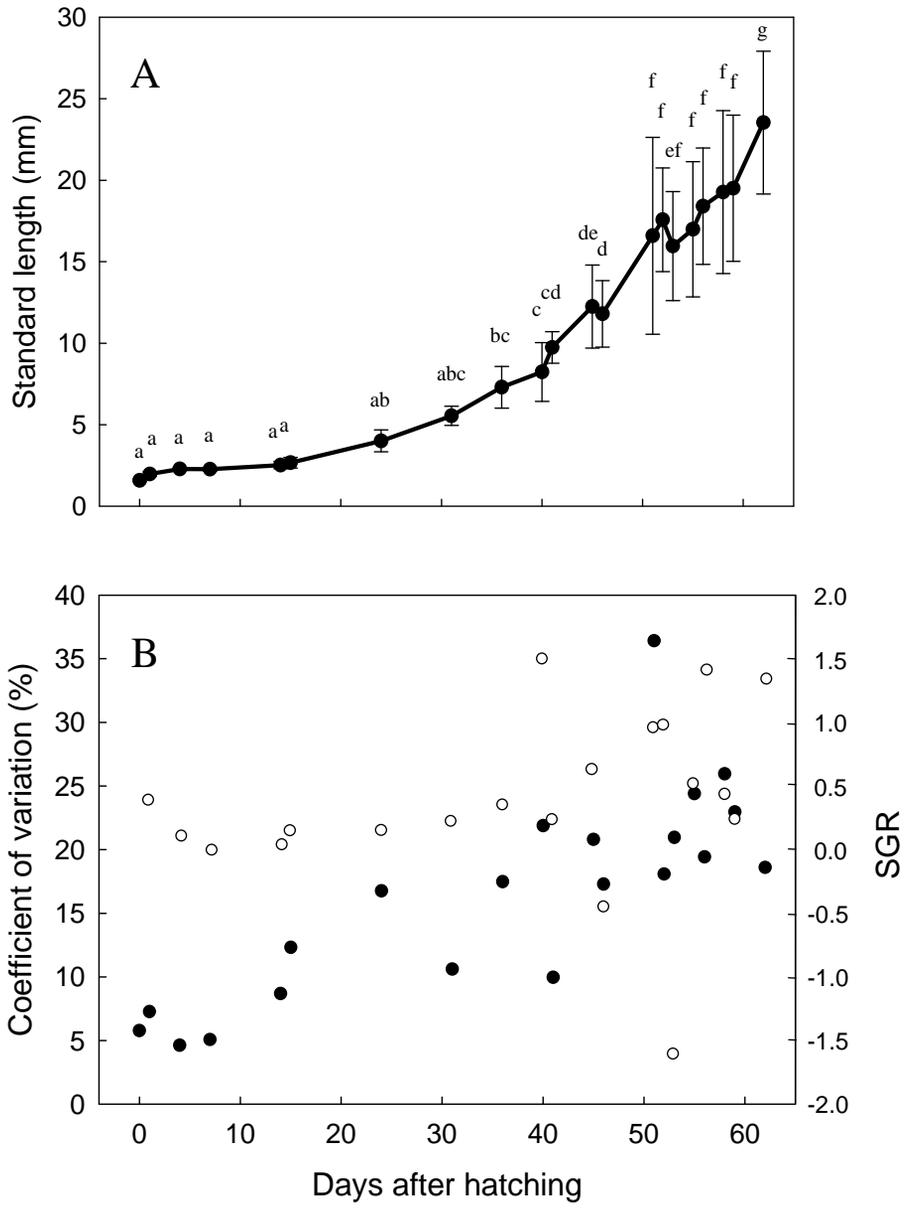
Fig. 5. Changes in aggressive behavior of seven-band grouper (n=2-4). Dots indicate the mean chase frequency and bars indicate standard deviations. Significant differences are indicated by alphabets (Tukey-Kramer test, $p<0.05$, a>b>c).

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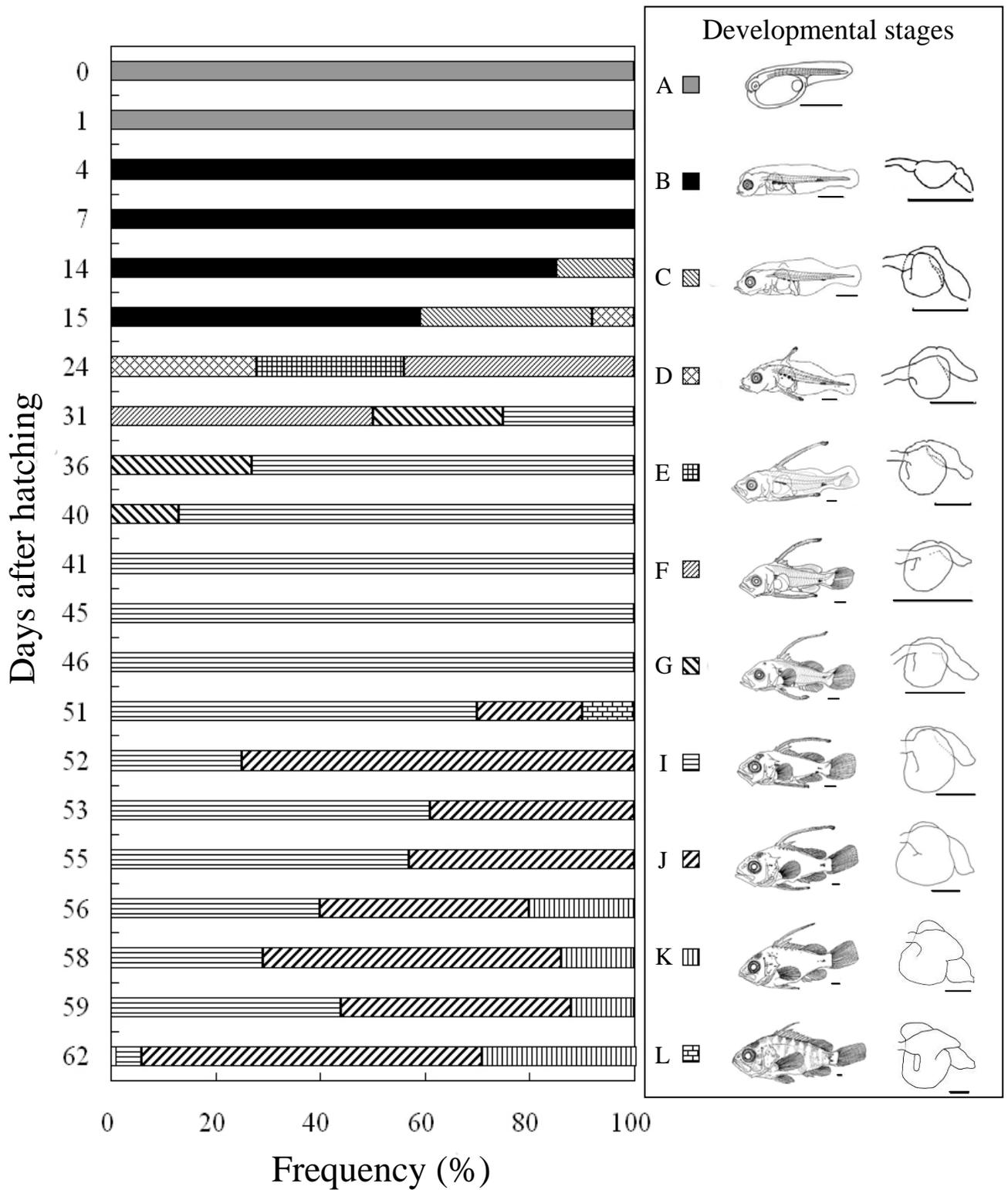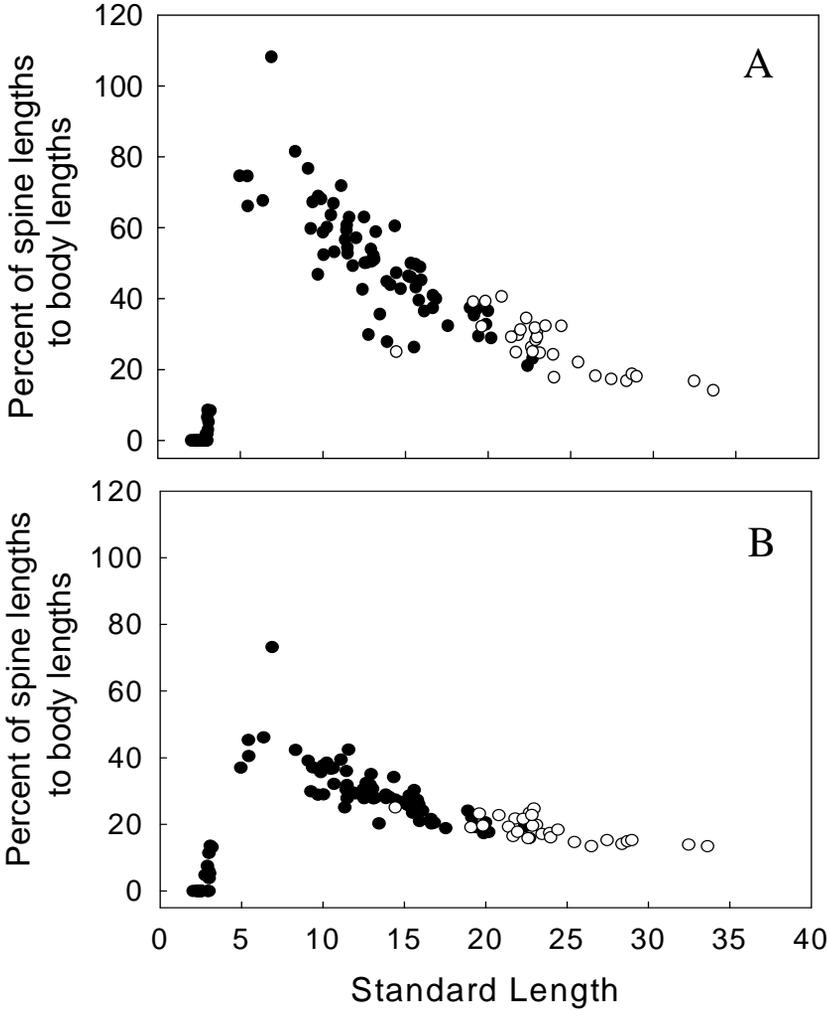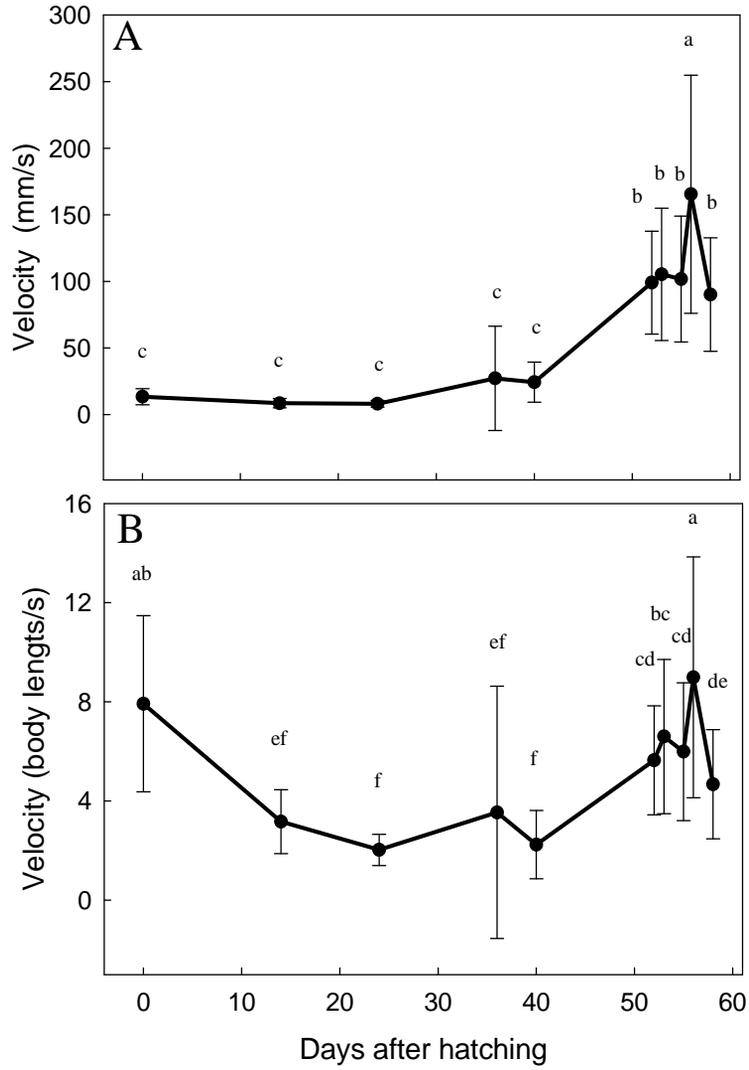
Fig. 2 Sabate et al., 2009

Fig. 3 Sabate et al., 2009

Fig. 4 Sabate et al., 2009

Fig. 5 Sabate et al., 2009