飼育下におけるサラサハタCromileptes altivelisの卵の観察

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Short Paper

Observation on Eggs of Mouse Grouper, *Cromileptes altivelis* under Rearing Conditions

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Abstract: Artificially collected eggs of *Cromileptes altivelis* were observed before and after fertilization. Newly ovulated eggs (125 g, 1,995 eggs/g) were stripped from a female (1.2 kg). Immediately after fertilization, the eggs absorbed water and became fully spherical with a hard covering membrane. Each egg had an oil globule. The diameter of the fertilized eggs ranged from 0.80 to 0.86 mm. The egg development was also observed. The eggs hatched from 23 h 39 min to 28 h after fertilization at 26.3-27.8℃. The fertilization and hatching rates were 90.5% and 73.8%, respectively.

Key words: *Cromileptes altivelis*, eggs, seed production, egg development

Mouse grouper, *Cromileptes altivelis* (Fig. 1) is a Serranidae and known as "Barramundi Cod" or "Humpbacak Seabass" in English, "Sarasahata" in Japanese, and "Ikan Kubin" in Malay[1-5]. The fish is distributed from southern Japan to the Indian Ocean and northern Australia[1-3]. The wholesale price of exports from Sabah, Malaysia to Hong Kong is RM100-180/kg (US$26-47/kg), nearly ten times more expensive than seabass, *Lates calcarifer* which is the most popular mariculture fish in Southeast Asia[4-6]. In Malaysia and most neighbouring countries, *C. alitivelis* is caught using destructive methods such as poisoning or bombing, resulting in severe depletion of this fish. However, there is no aquacultural production of *C. alitvelis* in Malaysia[7]. Due to its high export value, fish farmers have shown interest in developing *C. alitivelis* culture, but shortage of seed is the main impediment. In an attempt to establish a seed production technique for *C. alitivelis*, the authors collected eggs and sperm under rearing conditions and observed the eggs before and after fertilization for the first time in Malaysia.

The experiment was conducted at Kinarut Fish Hatchery managed by Borneo Marine Research Institute of Universiti Malaysia Sabah (UMS) in April 2000. A female (1.2 kg) and two male (2.6 kg and 3.1 kg) brood fish of *C. alitivelis* were used for the experiment. The female had a soft, distended abdomen and white unripe eggs could be obtained by cannulation. The males could ooze milt with gentle pressure near the genital pore. These specimens were procured from the wild population in Sabah and cultured in net cages in UMS Dinawan Fish Farm (former Aqua-Vision Fish Farm) for 2-3 years.

For maturation, the female was given a 600-IU injection of HCG (human chorionic gonadotropin) in the abdominal cavity[6]. After the HCG injection, the female and males were stocked in a tank (245×121×77 cm) containing 2 k*l* seawater. The water temperature, dissolved oxygen (DO), and pH during the maturation period ranged from 26.3-27.2℃, 5.8-6.3 mg/*l*, and 7.8-8.1, respectively.

Forty h and 30 min after the HCG injection, newly ovulated eggs were stripped out from the female into a bowl and weighed (A & D 1200G). One m*l* of unfertilized eggs was also weighed (A & D HF-400). The number of eggs was counted with the naked eye. After the egg stripping, sperm were collected from the males using a sperm collector[5,8]. The stripped eggs and sperm were mixed and artificially fertilized with some seawater[5]. Subsequently, the fertilized eggs were washed using a hand net in seawater and incubated in a tank (110 cm in diameter, 90 cm in height) with 0.7 k*l* seawater. Before the egg stripping and sperm collection, the brood fish were anesthetized with benzocaine (Ethyl 4-Aminobenzonate).

The egg development was observed under a microscope (Nikon Eclipse E 600) and photographs were taken with a digital camera (Kodak DC280 ZOOM). Water temperature, DO, and pH during the incubation period ranged from 26.3-28.2℃, 6.3-6.5 mg/*l*, and 7.7-8.3, respectively.

Stripped egg mass was shiny white in colour and weighed 125 g. Under the microscope these eggs were transparent and unsettled spherical shape (Fig. 2). Each egg had an oil globule and a soft covering membrane. One m*l* of the eggs was weighed 1.004 g. The number of eggs was 2,003 eggs/m*l* or 1,995 eggs/g.

The morphological changes during the egg development are shown in Fig. 3A-S. Immediately after fertilization, the eggs absorbed water and acquired a spherical shape with a hard covering membrane (Fig. 3A). Twenty-six min after fertilization (0:26 h), the blastodisk appeared and at 0:48 h, the first cleavage occurred (Fig. 3B). The fertilization rate at 2-cell stage was 90.5%. During the 2-cell stage, the eggs and their oil globules measured 0.83 (n=10, range 0.80-0.86) mm and 0.17 (n=10, range 0.16-0.18) mm in diameter, respectively. The 4-cell stage (Fig. 3C), 8-cell stage (Fig. 3D), 16-cell stage, and 32-cell stage (Fig. 3E) occurred in 2 h. Then morula (Fig. 3F), blastula, and gastrula (Fig. 3G) stages developed in that order in 3-8 h. At 9:41 h, embryo formation commenced (Fig. 3H) and at 12:27 h, head and 5-myomeres formed and Kupffer's vesicle appeared (Fig. 3K). Optic and lens vesicles were visible at 13:52 and 19:26 h, respectively (Fig. 3L and 3N). At 21:00 h, the embryo commenced movement (Fig. 3O) and at 22:03 h, a heart was formed and started moving (Fig. 3P). After this stage, the embryo exhibited active movement. At 22:38 h, otocyst vesicles appeared (Fig. 3Q). Hatching began at 23:39 h (Fig. 3R) and finished at 28:00 h before eye pigmentation. The hatching glands were not observed under the microscope. Newly hatched larvae (Fig. 3S) floated and turned up the yolk sac on the water surface without aeration. The hatching rate from the 2-cell stage was 73.8%.

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Fig. 1. Adult female fish of *Cromileptes altivelis*. 1.5 kg in body weight, 40 cm in total length.

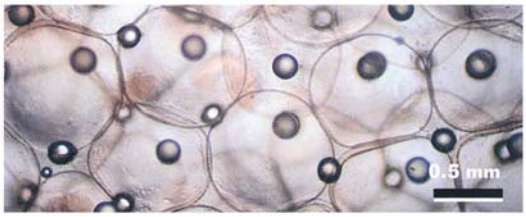

Fig. 2. Stripped eggs from the female of *C. altivelis* before fertilization.

The diameter of the fertilized eggs (0.80-0.86 mm) was similar to observations by Tang *et al*. (0.80-0.83 mm)[9] and also similar to same Serranidae of *Epinephelus tauvina* (0.71-0.90 mm)[10] and *E. akaara* (0.71-0.77 mm)[11]. However, the diameter was smaller than *E. striatus* (1.02 mm)[12]. The diameter of the oil globules (0.16-0.18 mm) was similar to those observed on *E. tauvina* (0.16-0.20 mm)[10] and *E. akaara* (0.13-0.16 mm)[11] and smaller than *E. striatus* (0.22 mm)[12]. The development of fertilized eggs and hatching time depend on water temperature during incubation period. In this study, the eggs hatched during 23:39 to 28:00 h at 26.3-28.2℃, which was similar to observations by Tang *et al*. (22 h at 28.4℃)[9] and the egg development was similar to the above Serranids. This experiment demonstrated that 125 g eggs were stripped from a 1.2 kg female and yielded around 170,000 newly hatched larvae. From this result, we can expect to obtain approximately 140,000 newly hatched larvae per kg female of *C. altivelis* under artificial conditions.

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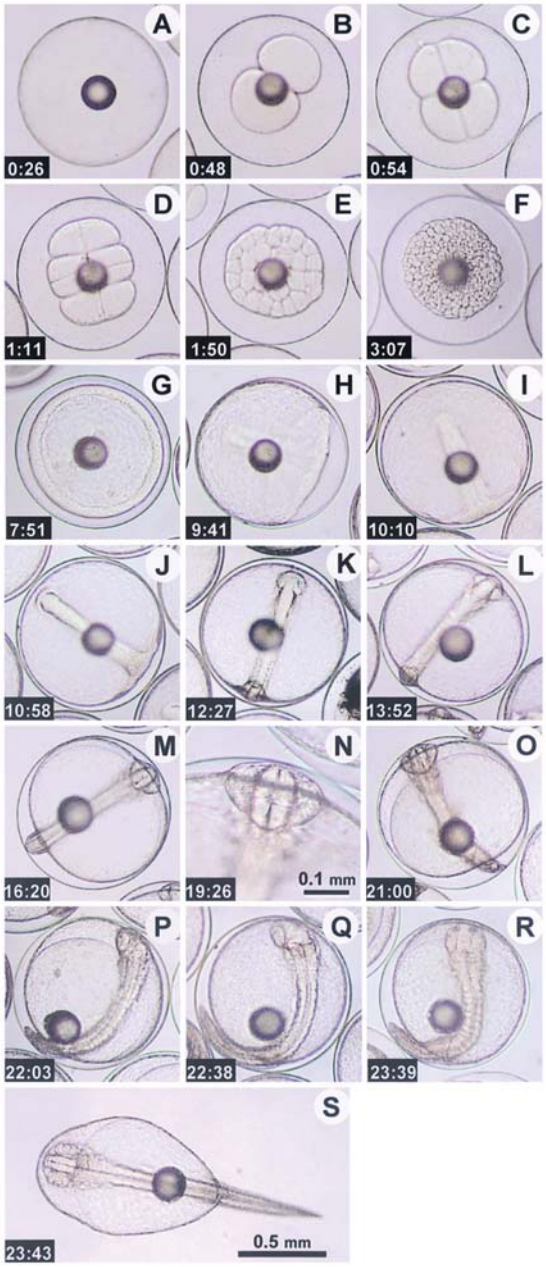

Fig. 3. Egg development of *C. altivelis*. **A**, fertilized egg; **B**, 2-cell stage; **C**, 4-cell stage; **D**, 8-cell stage; **E**, 32-cell stage; **F**, morula stage; **G**, gastrula stage; **H**, embryo formation commenced; **I**, blastopore nearly closed; **J**, blastopore completely closed; **K**, head and 5-myomere formed, Kupffer's vesicle appeared; **L**, optic vesicles appeared; **M**, tail separated from the yolk sac; **N**, lens vesicles appeared; **O**, embryo commenced moving; **P**, heart formed; **Q**, otocyst vesicles appeared; **R**, hatching started; **S**, hatched larva; numbers show hours and minutes after fertilization; the scale (0.5 mm) are all for **A** to **S** without **N**.

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