Aquacult. Sci. **68**(3), 235–242 (2020)

Spawning characteristics of a lunar-synchronized spawner, the honeycomb grouper *Epinephelus merra*, under artificial conditions

Takafumi AMAGAI[1, 2], Ryosuke MURATA[2] and Kiyoshi SOYANO[1, 2,*]

Abstract: The honeycomb grouper, which is a lunar-synchronized spawner, is an important fishery target species in Okinawa. In this study, to clarify the spawning characteristics of this species, we observed mass spawning (4 or 6 males and 20 or 22 females in 3-t tank) and single-pair spawning (one male and one female in a 200 *l* tank) using rearing experiments. The mass spawning results indicated spawning started 3 days after the full moon and continued for several days. However, the single-pair spawning results showed the starting date of spawning varied between individuals. The spawned eggs in 1 female were confirmed for 4 consecutive days (20,000–70,000 per day). The egg buoyancy, fertilization, and hatching rates were high in single-pair spawning until day 3. However, the number of spawned eggs, buoyancy, fertilization, and hatching rates at day 4 were extremely low, suggesting that actual spawning occurred for 3 days.

Key words: Honeycomb grouper, Spawning characteristics, Fecundity, Lunar cycle

Groupers (Serranidae) are a high commercial value fish and target species for fisheries among Asian and Oceanian coastal countries (Heemstra and Randall 1993; Craig et al. 2011). Recently, many grouper species have been considered endangered and are being targeted for stock management owing to ensure sustainable resource utilization (Sadovy de Mitcheson et al. 2013). Therefore, the artificial seed production and culture of groupers are being developed. In some cultured groupers, fertilized eggs were obtained through natural spontaneous spawning of captive fish (Tucker 1994) or by artificial insemination using fish treated with maturation-related hormones (Suquet et al. 1995; Linhart et al. 2006; Ninhaus-Silveira et al. 2006). However, this has not resulted in completely stable seed production. A reason is that the reproductive and spawning characteristics of groupers are not well understood.

The following are grouper maturation and spawning characteristics: Most grouper species are protogynous hermaphrodites, which primarily mature as females and later change sex to males (Erisman et al. 2009). There are many other interesting factors, especially during the spawning season. Among these, behavioral characteristics are considered important for spawning and next generation production. Groupers show characteristic spawning behaviors, such as spawning migrations to specific spawning grounds (Colin et al. 1987; Aguilar-Perera and Aguilar-Dávila 1996; Erisman et al. 2007), aggressive behavior between males (Luckhurst 2010; Kline et al. 2011), and courtship between males and females at spawning grounds (Johannes et al. 1999; Erisman and Allen 2005; Nemeth et al. 2006; Samoilys et al. 2014; Rowell et al. 2019). However, it is unknown whether the reproductive physiological changes caused by

Received 10 December 2019; Accepted 16 June 2020.

[1] Graduate School of Fisheries and Environmental Sciences, Nagasaki University, 1-14, Bunkyo-machi, Nagasaki 852-8521, Japan.

[2] Institute for East China Sea Research, Organization for Marine Science and Technology, Nagasaki University, 1551-7, Taira-machi, Nagasaki 851-2213, Japan.

[*] Corresponding author: Tel, (+81) 95-850-7701; Fax, (+81) 95-840-1881; E-mail, soyano@nagasaki-u.ac.jp (K. Soyano).

these behavioral and ecological changes occur in artificial environments. Furthermore, the spawning characteristics, such as the number of spawnings and spawning intervals are unknown.

The honeycomb grouper *Epinephelus merra* is widely distributed in coastal shallow seawater around Asia and Oceania (Heemstra and Randall 1993), and is the most abundant grouper species of the coral lagoons in Okinawa Island, Japan. In Asian countries, the honeycomb grouper is caught by fishermen for live reef fish food trade (LRFFT), and the wild-caught juveniles are utilized for aquaculture (Liu and Yeeting 2011). In Okinawa, Japan, this species is utilized as a local seafood, but is not a target species for artificial seed production and aquaculture. However, the fishing pressure of the honeycomb grouper could increase in the future, because the trade area has been expanding, e.g., in the fish market of Hong Kong (Sadovy de Mitcheson et al. 2013). Over the past few years, although there is no statistical evidence and only results of interviews with fishermen, fishermen in Okinawa have felt that the population size of this species is considered to be rapidly decreasing and artificial seedling production is necessary. Therefore, we determined that the reproductive characteristics in artificial environments should be examined as essential information for seed production in this species. Interestingly, this species has been known as an experimental model for the reproductive biology of groupers because of the following characteristics: abundant distribution in shallow coral areas, small body size (up to 320 mm), and relatively brief maturation duration to males at < 5 years of age (Craig et al. 2011); detailed information on the reproductive biology of this species, e.g., reproductive cycle (Lee et al. 2002; Soyano et al. 2003), sex change (Nakamura et al. 2005), and spawning migration (Soyano and Nakamura 2006). This also indicates that this species can be used as a model species to understand grouper maturation.

In the present study, we used two different rearing experiments, mass and single-pair spawning. Fish management for artificial seed production was carried out by large-scale breeding to induce mass spawning. Even in natural environments, many grouper species form a group for spawning with many individuals (Aguilar-Perera and Aguilar-Dávila 1996; Samoilys et al. 2014). A mass spawning experiment was conducted to confirm the spawning status of the group spawning in an artificial environment. However, the spawning population contains multiple females. Since not all females spawn synchronously, it is necessary to know the spawning characteristics of each female. Therefore, we conducted single-pair spawning. These studies aimed to elucidate the following spawning characteristics of the honeycomb grouper: 1) timing of spawning initiation, 2) spawning period, 3) days of spawning per female during the spawning period, 4) number of spawned eggs, and 5) quality of spawned eggs.

Materials and methods

Rearing condition of fish for mass spawning experiment

A few days before full moon on May 27, 2002, adult honeycomb grouper individuals were captured by hook and line at a coral reef lagoon around Sesoko Island and Nakijin in Okinawa, and transported to Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Motobu Town, Okinawa, Japan. Both adult males and females were reared in a 3-t fiber reinforced plastic (FRP) outdoor tank (round shape) with polyvinyl chloride pipes as a shelter and artificial plastic plants

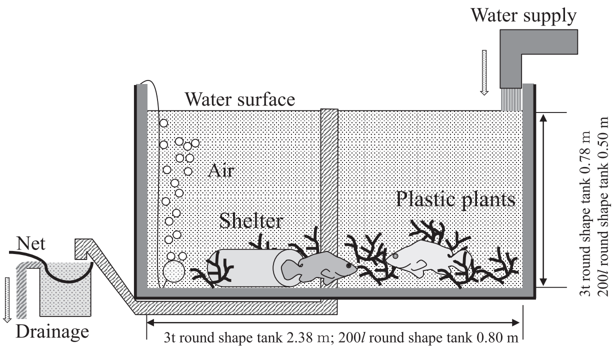

Fig. 1. The experiment tanks (3t and 200 *l*) for egg collection. The egg collecting net was under the overflow outlet. The aeration and shelter (polyvinyl chloride pipes as a shelter and artificial plastic plants resembling coral branch) were set in all tanks.

Table 1. Period of spawning, number of eggs, and buoyancy, fertilization, and hatching rates in each experimental tank

Month	Fish number		Spawning day and period (DAFM)	Total number of egg	Rate (%)		
	Male	Female			Buoyancy	Fertilization	Hatching
May	6	22	May 30-June 2 (3-6)	3,748,250	64.8	100	98.7
June	4	20	June 30 and July 1 (4, 5)	340,750	42.8	N	N

DAFM; day after full moon, N; not measured

resembling coral branches (Fig. 1). The sex of the experimental fish was determined by stripping the abdomen to confirm spermiation immediately after the fish were transported to Sesoko Station. The fish were fed small pieces of fish fillet and dry pellets, Otohime EP4 (Marubeni Nisshin Feed Co., Ltd., Japan) 3 or 5 days a week until satiation. In the experiment in May, 6 males and 22 females were kept in a round-shape indoor tank (Table 1) that was set up near the windows to keep the natural photoperiod and lunar conditions. In the experiment in June, 4 males and 20 females from the fish used in May were used under the same conditions in May. The rearing water was sand filtrated natural seawater from near the Sesoko Station. The water temperature was measured at 10:00 am daily. These rearing experiments were performed under natural photoperiod and temperature (24.5–26.0℃). The water overflow from the rearing tank was passed through a mesh net (50 × 50 μm) for egg collection. These eggs were confirmed at 10:00 am daily during the rearing experiment. Simultaneously, the sunken eggs in the bottom of tank were collected using a scooping net. The body weight (BW) and total length (TL) of females obtained prior to the experiment were 41–186 g and 145–223 mm, and those of male were 113–214 g and 194–250 mm, respectively. The required number of individuals for the experiment were selected from these individuals. However, the BW and TL of each individual was not measured in this experiment when placed in the experimental tank, because it was necessary to reduce stress so the fish could be used for different artificial seed production experiments.

Rearing condition of fish for the single pair spawning experiment

Experimental fish were captured at the coral

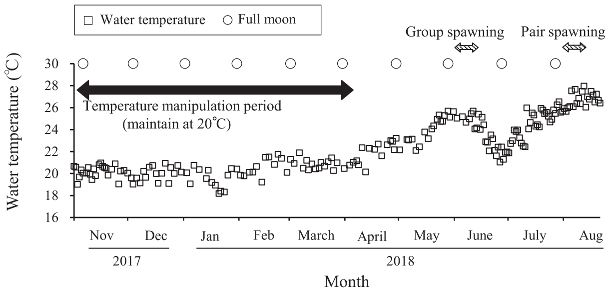

Fig. 2. Changes in water temperature in rearing tanks. Quadrangle shows the water temperature in the rearing tank, black double arrows show temperature settings in the heater, white double arrows show the single-pair spawning experiment period, open circle shows full moon days.

reef lagoon, Nakijin, Okinawa in May 2017. On May 20, 21 fish were transported to the Institute for East China Sea Research, Nagasaki University, Nagasaki City, Japan by a shipping company. Then, to acclimate this environment for spawning the next year, the fish were reared in 3 t FRP outdoor tanks (round shape) until November 1, 2017. Then, to overcome the low water temperature period in winter, 4 males and 11 females were transferred and reared in a 500 l indoor tank (round shape) that was set up near the windows to ensure natural photoperiod and lunar conditions. The rearing water was sand filtrated natural seawater supplied from front of our institute and flowing to the tank (1 l/min) with aeration and a heater (500 W titanium heater and the controller, Nittokizai Company) to heat to 20℃ using until the end of March when the natural water temperature exceeded 20℃ (Fig. 2). During the rearing period from November to March, to maintain the water temperature at 20℃, the room temperature was maintained at 25℃ using an air conditioner. The water temperature gradually increased from April to June, when it reached a suitable spawning temperature of 25℃ in late May. Mass spawning occurred from May 30 to June 3, a few days after the full moon. On June

19, one female and one male were placed into a 200 *l* tank to observe their spawning characteristics after the determination of sex of the experiment fish using the same method of the mass spawning experiment (Fig. 1). Four sets of experimental tanks were prepared in this study. Unfortunately, owing to a malfunction of the experimental equipment, the water temperature temporarily dropped in June. Therefore, at the end of June, the fish were not able to spawn. The water temperature was readjusted and a pair spawning test was conducted in July. From July 12 to the end of August, the water temperature was kept between 24 and 28℃ using the air conditioner. Spawning occurred between July 31 and August 6 immediately after the full moon on July 28 at a water temperature of 25.6–27.2℃. The spawned eggs were collected in the same way as described in the mass spawning experiment. Throughout the rearing period from May 2017 to August 2018, the fish were fed dry pellets, Otohime EP4 (Marubeni Nisshin Feed Co., Ltd., Japan) 3 or 5 days a week until satiation. The BW and TL of each pair used in the experiment measured at the start of the experiment are shown in Table 2.

Measurement of buoyancy, fertilization, and hatching rates

The volume of eggs collected from rearing tanks was measured in a measuring cylinder, and the number of eggs per milliliter was counted under a stereoscopic microscope (mean = 3,580.5 eggs/m*l*, n = 10). The volume of buoyant and sunken eggs was measured in the measuring cylinder. The number of eggs was estimated by multiplying the number of eggs per milliliter by the volume of eggs. Approximately 100 eggs were selected from floating eggs at random and the stage of embryonic development was observed under the stereo microscope to confirm fertilization. Then, the fertilized eggs were counted and the fertilization rate was calculated. To calculate the hatching rate, approximately 50 eggs were randomly selected from the floating eggs and were incubated in the 500 mL plastic bottle with seawater at an ambient water temperature (25.6–26.8℃) for 24 hours. Then, the hatched larvae were counted under the stereo microscope and the rate of hatching larva to collected eggs was calculated. Unfortunately, the fertilization and hatching rates were not determined in June, owing to limitations of the experimental facility.

Experimental animal care

All experimental procedures involving animals were conducted in compliance with the Animal Care and Use Committee of the Institute for East China Sea Research, Nagasaki University, Japan (Permit Number #15-06).

Results

Spawning characteristics of mass spawning

Mass spawning was conducted and spawned eggs were observed for 4 days from May 30 to June 2 after the full moon (May 27) and for 2 days on June 30 and July 1 after the full moon (June 26) (Table 1). The average buoyancy

Table 2. Observation of spawned eggs in the honeycomb grouper *Epinephelus merra* of single-pair rearing

Tank	Experimental fish				Spawning day (DAFM)										
	Sex	N	TL (mm)	BW (g)	July 28 (0)	July 29 (1)	July 30 (2)	July 31 (3)	Aug 1 (4)	Aug 2 (5)	Aug 3 (6)	Aug 4 (7)	Aug 5 (8)	Aug 6 (9)	Aug 7 (10)
Pair 1	M	1	245	177.5				+	+	+	+				
	F	1	163	52.9											
Pair 2	M	1	268	265.3				+	+	+	+				
	F	1	220	150.3											
Pair 3	M	1	248	227.0					+	+	+	+			
	F	1	171	92.1											
Pair 4	M	1	223	189.7							+	+	+	+	
	F	1	157	74.6											

+; egg collected in rearing tank, M; male, F; female, TL; total length, BW; body weight, DAFM; day after full moon.

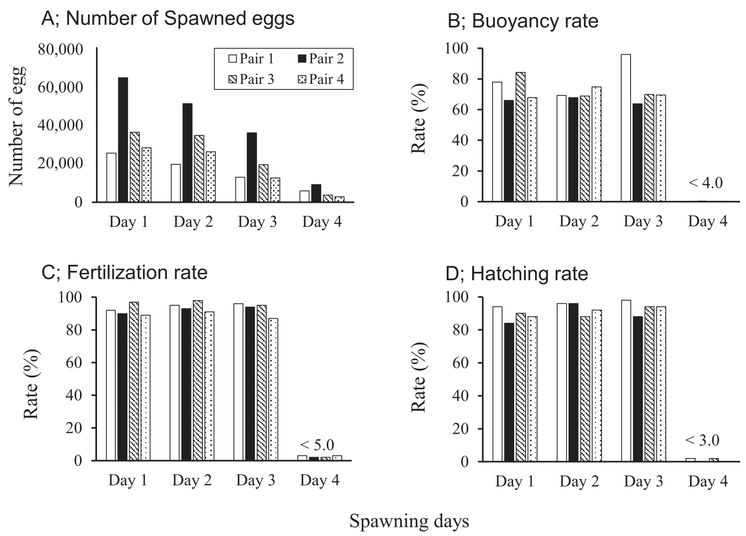

Fig. 3. Changes in the evaluation items of single-pair spawning under artificial conditions. A, the number of spawned eggs in rearing tanks; B, egg buoyancy rate; C, fertilization rate; D, hatching rate. White, black, oblique, and spotted columns show pair 1, 2, 3, and 4, respectively.

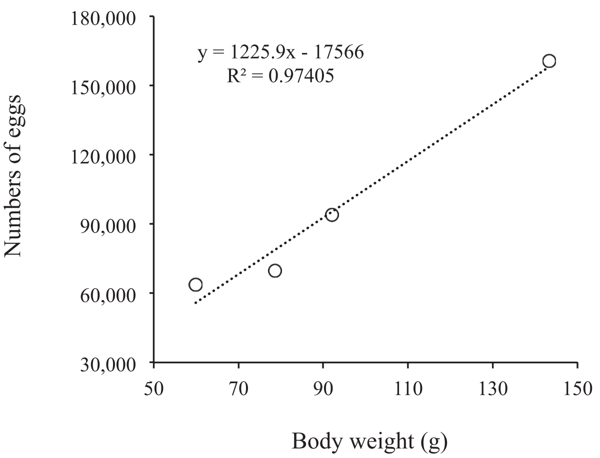

Fig. 4. Relationship between body weight and numbers of eggs spawned by each female. Total number of eggs; total amount of eggs for consequent 4 spawning days.

rate of spawned egg over 4 days was 64.8% and 42.8% in May and June, respectively. The average fertilization and hatching rates of spawned egg over 4 days in May were 100% and 98.7%, respectively.

Spawning characteristics of single-pair spawning

In the single-pair experiment, the spawned eggs were observed from days 3 (July 31) to 9 (August 6) after the full moon (July 28) in all of the 4 pairs (Table 2). All pairs spawned for 4 consecutive days. However, the start date of spawning was different for each pair; therefore, the spawning period of the 4 pairs as a whole was 7 days.

The number of spawned eggs was 20,000–70,000 on day 1 and decreased gradually each day thereafter (Fig. 3). However, the numbers varied for each pair. The largest number of eggs was released by the largest female with a TL of 200 mm. The number of eggs confirmed on day 4 was very low in all groups. The buoyancy, fertilization, and hatching rates in each pair was consistent at > 60%, > 80%, and > 80% from day 1 to 3, respectively, and were extremely low on day 4 (< 0.5%). The relationship between BW and total number of eggs spawned in each female showed a strong positive correlation (Fig. 4).

Discussion

The honeycomb grouper is a lunar-synchronized spawner (Lee et al. 2002; Soyano et al. 2003). In the present study, lunar-synchronized spawning behavior in the honeycomb grouper reared under artificial conditions was confirmed. In particular, the fish reared in tanks in Nagasaki after transportation from Okinawa maintained the maturation and spawning rhythm related to the lunar cycle even after one year, as described in previous reports (Soyano et al. 2003); the fish spawned for several days after the full moon. Although flowing natural seawater was used in the rearing experiment, fish could not experience tidal rhythms; therefore, the lunar rhythm for maturation and spawning in this species is thought to be induced by the change of lunar light, and not tidal rhythms. Although lunar-synchronized maturation and spawning has been observed in some groupers (Tucker et al. 1996; Teruya et al. 2008; Starr et al. 2018), there have been no scientific reports, to the best of our knowledge, showing this characteristic has been maintained over time in captivity.

The most notable results of this study were the successful spawning and egg fertilization under artificial conditions without maturation-related hormone treatment, and the accurate assessment of female spawning numbers in the single-pair spawning experiment. In previous studies, ovulation induction and spawning of captive groupers were investigated to develop artificial seed production techniques, suggesting egg quality varied with several factors, such as season, rearing condition, and induced spawning methods (Tucker 1994). It is difficult to obtain fertilized eggs from groupers under artificial conditions without hormone treatment. Therefore, in many grouper species, hormone [e.g., human chorionic gonadotropin (HCG), luteinizing hormone analog (LH-RHa)] treatment and artificial insemination has been conducted to obtain fertilized eggs e.g., longtooth grouper (Imaizumi et al. 2005), sevenband grouper (Soyano et al. 2008), etc. These species are classified as medium to large groupers. However, artificial seed of small groupers, such as the blacktip grouper, has been obtained easily by natural spawning without hormone treatment and artificial insemination (Kawabe et al. 2000). For this to be successful, it is necessary for the rearing tank to have sufficient space for spawning behavior. During grouper spawning, the males chase the females (Okumura et al. 2002), which requires adequate space (Okumura et al. 2003). It is possible that groupers reproduced via hormonal administration and artificial insemination only would be able to spawn naturally if sufficient space was prepared. The honeycomb grouper used in this study is one of the smallest grouper species. Therefore, it appears that this species was able to spawn without hormonal treatment because there was sufficient space for spawning behavior, even in a 200 *l* tank. The honeycomb grouper can be used as a good model for the final maturation and spawning experiment of groupers because this species spawns in a small aquarium with reproducibility.

The fertilization rate of the present study was higher that of previous studies of the red spotted grouper (Okumura et al. 2003) and blacktip grouper (Kawabe et al. 2000) by natural spawning. According to Okumura et al. (2003), the large volume tank improved the fertilization rate in the red spotted grouper. These results suggested that good results were obtained in the present study because sufficient space was secured; a 3 t tank was adequate for the honeycomb grouper.

The number of spawned eggs, buoyancy rate, fertilization rate, and hatching rate of spawned eggs obtained by hormone administration and artificial insemination are lower than those of natural spawning (Okumura et al. 2002; Marino et al. 2003). No comparison of these factors was carried out between natural spawning and artificial insemination in the present study; however, this species did not require hormonal administration that has a possibility to suppress fertilization rate, hatching rate etc. These results showed that artificial seed can be produced by natural spawning using a small aquarium.

In the present study, we investigated the spawning characteristics of a single male and female pair in rearing conditions. In all of the four pairs, spawned eggs were observed for 4 consecutive days. The amount of spawned eggs was highest on the first day and decreased daily. The buoyancy, fertilization, and hatching rates were similar until day 3, but decreased sharply on day 4. The hatching rate was $< 3\%$ on day 4. From these results, it is possible that the spawned eggs were the eggs that had ovulated the previous day. These results indicated that the actual spawning continued for 3 days in the female of each pair, which was consistent with the histological observations (Amagai et al. unpublished data). The spawning characteristics of the females were the same for all 4 pairs. However, the start date of spawning was different for each pair. There was a three-day gap in the start of spawning between the earliest and latest pairs. The spawning period when spawning migration occurs is estimated to be approximately 5 days in the honeycomb grouper (Soyano and Nakamura 2006). However, this is possibly because individuals with different starting dates were grouped together. The different starting of spawning by lunar-synchronized spawners in a rearing condition was reported in Nassau grouper, *E. striatus* (Tucker et al. 1996) and camouflage grouper, *E. polyphekadion* (Teruya et al. 2008), although the mechanisms causing the difference is unknown.

The number of spawned eggs in the single-pair experiment was different in each pair. These differences were dependent on body size. It is known that large individuals release many eggs (Okumura et al. 2002). The gonadosomatic index (GSI), which is the proportion of ovary to body weight, is roughly determined for each species. Regarding the honeycomb grouper, the GSI immediately before spawning is approximately 10%–13% (Lee et al. 2002; Soyano and Nakamura 2006). Consequently, the difference in the amount of spawned eggs is strongly dependent on female body size.

In conclusion, lunar-synchronized spawning in the honeycomb grouper was confirmed under artificial conditions and spawning continued for 3 days in one female. Such information is useful for resource management and aquaculture of the honeycomb grouper, which is an important coastal fishery species in Okinawa.

Acknowledgments

We would like to thank Prof Masaru Nakamura for valuable advice and Dr. Yoshikatsu Nakano, and Mr. Shigeo Nakamura for their valuable assistance in rearing the fish and collecting the samples. We also would like to thank Editage (www.editage.jp) for English language editing.

References

Aguilar-Perera, A. and W. Aguilar-Dávila (1996) A spawning aggregation of Nassau grouper *Epinephelus striatus* (Pisces: Serranidae) in the Mexican Caribbean. *Environ. Biol. Fishes*, **45**, 351–361.

Colin, P. L., D. Y. Shapiro and D. Weiler (1987) Aspects of the reproduction of two groupers, *Epinephelus guttatus* and *E. striatus* in the West Indies. *Bull. Mar. Sci.*, **40**, 220–230.

Craig, M. T., Y. J. Sadovy de Mitcheson and P. C. Heemstra (2011) Grouper of the World: A Field and Market Guide. NISC (Pty) Ltd, Grahamstown, South Africa, 424 pp.

Erisman, B. E. and L. G. Allen (2005) Color patterns and associated behaviors in the kelp bass, *Paralabrax clathratus* (Teleostei: Serranidae). *Bull. South. Calif. Acad. Sci.*, **104**, 45–62.

Erisman, B. E., M. L. Buckhorn and P. A. Hastings (2007) Spawning patterns in the leopard grouper, *Mycteroperca rosacea*, in comparison with other aggregating groupers. *Mar. Biol.*, **151**, 1849–1861

Erisman, B. E., M. T. Craig and P. A. Hastings (2009) A phylogenetic test of the size - advantage model: evolutionary chnges in mating behavior influence the loss of sex change in a fish lineage. *Am. Nat.*, **174**, E83–E99.

Heemstra, P. C. and J. E. Randall (1993) FAO Species Catalogue, Vol.16. Groupers of the World. FAO Fisheries Synopsis, **16**, 1–382.

Imaizumi, H., T. Hotta and H. Ohta (2005) Cryopreservation of kelp grouper *Epinephelus bruneus* sperm and comparison of fertility of fresh and cryopreserved sperm. *Aquacult. Sci.*, **53**, 405–411. (in Japanese with English abstract)

Johannes, R. E., L. Squire, T. Graham, Y. Sadovy and H. Renguul (1999) Spawning aggregations of groupers (Serranidae) in Palau. *Nat. Conserv. Mar. Res. Ser. Publ.*, No. 1.

Kawabe, K., K. Kato and J. Kimura (2000) Year round spawning of reared blacktip grouper, *Epinephelus fasciatus*, in Chichi-jima. *Aquacult. Sci.*, **48**, 467–473. (in Japanese with English abstract)

Kline, R. J., I. A. Khan and G. J. Holt (2011) Behavior, color change and time for sexual inversion in the protogynous grouper (*Epinephelus adscensionis*). *PLoS One*, **6**, e19576.

Lee, Y. D., S. H. Park, A. Takemura and K. Takano (2002) Histological observations of seasonal reproductive and lunar-related spawning cycles in the female honeycomb grouper *Epinephelus merra* in Okinawan waters. *Fish. Sci.*, **68**, 872–877.

Linhart, O., M. Rodina, M. Kocour and D. Gela (2006) Insemination, fertilization and gamete management in tench, *Tinca tinca* (L.). *Aquacult. Int.*, **14**, 61–73.

Liu, M. and B. M. Yeeting (2011) Epinephlus merra. In "*Groupers of the World: a Field and Market Guide*" (ed. by T. M. Craig, Y. J. Sadovy de Mitcheson and P. C. Heemstra), NISC (Pty) Ltd., Grahamstown, South Africa, pp. 191–193.

Luckhurst, B. E. (2010) Observations of a black grouper (*Mycteroperca bonaci*) spawning aggregation in Bermuda. *Gulf Caribb. Res.*, **22**, 43–49.

Marino, G., E. Panini, A. Longobardi, A. Mandich, M. G. Finoia, Y. Zohar and C. C. Mylonas (2003) Induction of ovulation in captive-reared dusky grouper, *Epinephelus* *marginatus* (Lowe, 1834), with a sustained-release GnRHa implant. *Aquaculture*, **219**, 841-858.

Nakamura, M., Y. Kobayashi, S. Miura, M. A. Alam and R. K. Bhandari (2005) Sex change in coral reef fish. *Fish Physiol. Biochem*., **31**, 117-122.

Nemeth, R. S., E. Kadison, S. Herzlieb, J. Blondeau and E. A. Whiteman (2006) Status of a yellowfin (*Mycteroperca venenosa*) grouper spawning aggregation in the US Virgin Islands with notes on other species. In "*Proceedings of the 57th Gulf and Caribbean Fisheries Institute*" GCFI, St Petersburg, FL, pp. 543-558.

Ninhaus-Silveira, A., F. Foresti, R. Veríssimo-Silveira and J. A. Senhorini (2006) Seminal analysis, cryogenic preservation, and fertility in matrinxã fish, *Brycon cephalus* (Günther, 1869). *Brazilian Arch. Biol. Technol.*, **49**, 651-659.

Okumura, S., K. Okamoto, R. Oomori and A. Nakazono (2002) Spawning behavior and artificial fertilization in captive reared red spotted grouper, *Epinephelus akaara*. *Aquaculture*, **206**, 165-173.

Okumura, S., K. Okamoto, R. Oomori, H. Sato and A. Nakazono (2003) Improved fertilization rates by using a large volume tank in red spotted grouper (*Epinephelus akaara*). *Fish Physiol. Biochem.*, **28**, 515-516.

Rowell, T. J., O. Aburto-Oropeza, J. J. Cota-Nieto, M. A. Steele and B. E. Erisman (2019) Reproductive behaviour and concurrent sound production of Gulf grouper *Mycteroperca jordani* (Epinephelidae) at a spawning aggregation site. *J. Fish Biol.*, **94**, 277-296.

Sadovy de Mitcheson, Y., M. T. Craig, A. A. Bertoncini, K. E. Carpenter, W. W. L. Cheung, J. H. Choat, A. S. Cornish, S. T. Fennessy, B. P. Ferreira, P. C. Heemstra, R. F. Myers, D. A. Pollard, K. L. Rhodes, L. A. Rocha, B. C. Russell, M. A. Samoilys and J. Sanciangco (2013) Fishing groupers towards extinction: A global assessment of threats and extinction risks in a billion dollar fishery. *Fish Fisheries.*, **14**, 119-136.

Samoilys, M. A., D. Macharia, J. Robinson, G. W. Maina and J. Bijoux (2014) Confirmed sighting of a spawning aggregation of the brown-marbled grouper, *Epinephelus fuscoguttatus*, in Kenya. *West. Indian Ocean J. Mar. Sci.*, **13**, 189-203.

Soyano, K., T. Masumoto, H. Tanaka, M. Takushima and N. Nakamura (2003) Lunar-related spawning in honeycomb grouper, *Epinephelus merra*. *Fish physiol. Biochem.*, **28**, 447-448.

Soyano, K and M. Nakamura (2006) Chapter 2: Lunar-related maturation and spawning migration in honeycomb grouper. In "*Aquatic Biotelemetry and Fishing Gear Telemetry*" (ed. by K. Yamamoto,T. Yamane, Y. Mitsunaga), Kouseisha Kouseikaku, Tokyo, pp. 22-30. (in Japanese).

Soyano, K., Y. Sakakura and A. Hagiwara (2008) Reproduction and larvaeculture of seven-band grouper, *Epinephelus septemfasciatus*, in Japan. In "*The Aquaculture of Groupers*" (ed. by I. C. Liao and E. M. Leaño), Asian Fisheries Society, World Aquaculture Society, the Fisheries Society of Taiwan and National Taiwan Ocean University, pp. 1-27.

Starr, R. M., E. Ballesteros, E. Sala and J. M. Llenas (2018) Spawning behavior of the tiger grouper (*Mycteroperca tigris*) in a Caribbean atoll. *Environ. Biol. Fishes*, **101**, 1641-1655.

Suquet, M., R. Billard, J. Cosson, Y. Normant and C. Fauvel (1995) Artificial insemination in turbot (*Scophthalmus maximus*): determination of the optimal sperm to egg ratio and time of gamete contact. *Aquaculture*, **133**, 83-90.

Teruya, K., S. Masuma, Y. Hondo and K. Hamasaki (2008) Spawning season, lunar-related spawning and mating systems in the camouflage grouper *Epinephelus polyphekadion* at Ishigaki Island, Japan. *Aquacult. Sci.*, **56**, 359-368.

Tucker, Jr. JW. (1994) Spawning by captive serranid fishes: a review. *J. World Aquacult. Soc.*, **25**, 345-359.

Tucker, Jr. J. W., P. N. Woodward and D. G. Sennett (1996) Voluntary Spawning of Captive Nassau Groupers Epinephelus striatus in a Concrete Raceway. *J. World Aquacult. Soc.*, **27**, 373-383.

月周産卵魚カンモンハタ *Epinephelus merra* の飼育下での産卵特性

天谷貴史・村田良介・征矢野　清

沖縄地方では重要な漁業対象種とされているカンモンハタの産卵特性を，雄 4 と雌20尾または雄 6 尾と雌22尾による飼育による集団産卵と雌雄 1 尾ずつ飼育によるペア産卵によって調べた。集団産卵では，満月の 3 日後あるいは 4 日後より数日間にわたって産卵が確認された。しかしペア産卵の結果，産卵の開始日は個体によって異なっている（満月の 3 日後から 6 日後）ことがわかった。ペア産卵群では 4 日間連続して放卵された卵が確認された。1 尾の雌の産卵量は，1 日あたり2,000粒から7,000粒であったが，4 日目には急減した。浮上卵率，受精率，孵化率は産卵開始から 3 日目までは高かったが，4 日目のそれらは極めて低かった。以上の結果より，本種の産卵は満月の 3 日目より開始され，1 尾の雌が 4 日間連続で産卵するが，実質的な産卵は 3 日間であることがわかった。