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Induced Spawning of Two Western Tropical Pacific Groupers, *Plectropomus areolatus* and *Epinephelus fuscoguttatus*, in Palau

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Abstract - In May and June 1991, 10 squaretail coral trout (*Plectropomus areolatus*) females ovulated 34-36 hours after the first of two intramuscular injections of human chorionic gonadotropin (HCG), producing 4,001,000 eggs; four fish later released an additional 1,792,000 eggs, and another 1,630,000 eggs were found in the holding tank. Five females ovulated naturally several hours after capture (about 12 hours before the new moon in June), producing 856,000 eggs. In June, three brown-marbled grouper (*Epinephelus fuscoguttatus*) females ovulated 37-39 hours after the first of one or two HCG injections, producing 5,035,000 eggs, with another 42,000 found in the tank. Coral trout egg diameter range was 687-814 μm (mean 754 μm) and oil globule diameter range 170-257 μm (mean 213 μm). Brown-marbled grouper egg diameter range was 743-826 μm (mean 789 μm) and oil globule diameter range 187-251 μm (mean 215 μm). In most cases, fertilization and hatching rates were high. Induced spawns produced 1,755,000 coral trout and 3,791,000 brown-marbled grouper hatchlings.

Squaretail coral trout (*Plectropomus areolatus*) and flowery cod or brown-marbled grouper (*Epinephelus fuscoguttatus*) are two of the main groupers harvested in Palau and are also important in other fisheries of the western tropical Pacific. Squaretail coral trout reach about 70 cm and range from the Marshall Islands and Samoa to the Maldives and Red Sea (Randall et al. 1990). This species has not been spawned in captivity or reared before, but *Plectropomus leopardus* has (Teruya et al. 1992; Masuma et al. 1993). Brown-marbled groupers reach at least 90 cm and range from the Marshall Islands and Phoenix Islands to East Africa and the Red Sea. Spawning and rearing research has been done with this species (Kohno et al. 1990a; Lim et al. 1990; Supriatna and Kohno 1990; Mayunar et al. 1991; Muchari et al. 1991; Maneewong and Hazza 1992), but with induced spawning reported for only one female (Kohno et al. 1990b). During a study in Palau (May-June 1991), we used human chorionic gonadotropin (HCG) to induce ovulation in these two species and obtained eggs of high viability.

All broodfish were caught in or near Ulong Channel, a well-known grouper spawning aggregation site along the southwestern edge of Palau's fringing reef system (Johannes 1981). The fish were taken to shaded 2,000 and 4,000-l fiberglass holding tanks at the Micronesian Mariculture Demonstration Center in Koror State. Females immediately were injected intramuscularly between the dorsal fin and lateral line with 700 IU HCG·kg^{-1} body weight; some received another full or half (350 IU·kg^{-1}) injection 24 hours later. Injections were not required for males of either species because enough running ripe individuals were available. Aerated water was supplied by an open seawater system. Throughout the study, water temperature was in the range 28.2-29.4°C and salinity was 34 ppt in broodfish tanks and incubation beakers.

Females were observed carefully for signs of hydration and ovulation. As in many other groupers, hydration is indicated by swelling of the abdomen, and ovulation by protrusion of the egg mass through the genital opening. The female does not swim smoothly, rests on the bottom more often, and could turn pale relative to its tankmates. When eggs were ready to flow freely, they were stripped, fertilized (dry method), washed and incubated in seawater passed through a felt filter bag rated at 10 μm. Egg number was estimated volumetrically. Fertilization and hatching rates were estimated with 100 eggs from each spawn in a 500-ml beaker of filtered seawater. Fertilization rate was determined as the percentage of normally developing eggs (of all released) at about 8 hours after fertilization. Hatching rate was determined as the percentage of eggs hatched of those fertilized. A sample of eggs from each spawn was preserved in 5% sodium borate-buffered formalin in 50% seawater. Two measurements from ten specimens per spawn were taken: (1) egg diameter (spherical), and (2) oil globule diameter (mean of short axis and long axis).

Plectropomus areolatus

To help predict spawning time, we made a preliminary collection of 39 squaretail coral trout at alternate sites, Ebiil and Yengel Passes along the northwestern reef, on 1 May (3 days after the full moon). Twenty female and 17 male broodfish were collected at Ulong Channel on 8 May, 13 May and 7 June.

No male:female size differential, which could indicate protogynous hermaphroditism in this species (Johannes 1988), was found in our small sample. The preliminary collection on 1 May contained 18 males (500-578 mm TL, mean 533), 18 females (420-590 mm TL, mean 529) and 3 undetermined (392-545 mm TL). The males were not running milt. All ovaries were similar - small and round with partly yolked oocytes.

In trial Pa-1, ovarian biopsies were taken with surgical polyethylene tubing (860 μm inside diameter). Five females were given two full injections of HCG. In trial Pa-2, biopsies were taken from females, and diameters of ten fresh oocytes from each were measured. Although oocytes were very mature, six females were given full doses of HCG during 1500-1800 on 13 May, and one was not injected. In trial Pa-3, biopsies were not taken and all eight females were given full injections. The next day, three were given full doses, two half doses, and three were not injected.

Trial Pa-1: Initial biopsied oocytes were clearing and most had single oil globules as in mature eggs. In the first set of spawns, a total of 2,428,000 eggs was obtained from the five fish (Table 1). Thirty-six hours later, they were stripped again, producing 1,792,000 more eggs, but none were viable. Another 1,630,000 eggs were found in the holding tank. The five females produced a total of 5,850,000 eggs by induced ovulation. Mean egg diameter was 744 μm. From 1.36 million eggs (females 1,2,4), 0.42 million larvae hatched.

Table 1. Trial Pa-1: Induced spawning of *Plectropomus areolatus*. Females were given two full injections (700 IU HCG·kg^{-1}) at 1350 on 8 May 1991 and 24 hours later (five males). Ovulation occurred 4.5 days before the new moon. Oil globule diameter range was 206-277 μm.

Female no.	Body weight (g)	Dosage (IU HCG)	Time to fertilization (hours)	Egg diameter (μm)	Number of eggs (1,000s)	Fertilization rate (%)	Hatching rate (%)
1	1,700	1,250	35	706	605	81	50
2	1,700	1,250	36	740	308	77	36
3	1,900	1,400	35	766	245	1	44
4	2,000	1,450	35	760	446	63	32
5	2,100	1,550	35	748	824	20	10

Trial Pa-2: Five of the seven females captured on 13 May, one day before the new moon, ovulated unexpectedly during the night, and the overripe eggs could not be fertilized the next morning (Table 2). Overall mean diameter of initial biopsied oocytes from the fish that ovulated was 675 μm. Mean diameter from the two that did not ovulate was 635 μm. Most biopsied oocytes from all seven fish had begun to clear and had only one oil globule. Follow-up biopsies from the two fish that did not ovulate revealed regressing and atretic oocytes. The five females produced a total of 856,000 eggs by natural ovulation.

Trial Pa-3: Of the eight females captured on 7 June, all five receiving two or one and a half doses ovulated high quality eggs; a total of 1,573,000 were stripped from the five fish (Table 3). No significant differences between the two

Table 2. Trial Pa-2: Natural ovulation of *Plectropomus areolatus* in Palau on 13 May 1991, about 12 hours before the new moon. All except number 9 had received one injection of 700 IU HCG·kg^{-1} a few hours before ovulation occurred (seven males). Eggs were overripe by the time the fish were examined the next morning. Reg = regressing.

Female no.	Body weight (g)	Initial oocyte diameter (μm)	Initial no. of oil globules	Number of eggs (1,000s)
6	1,000	589	1-6	187
7	1,250	748	4-10	35
8	1,300	572	1-4	245
9	1,400	793	1-4	303
10	1,850	618	1-4	Reg
11	1,950	674	1-4	86
12	1,950	652	1-4	Reg

Table 3. Trial Pa-3: Induced spawning of *Plectropomus areolatus*. All seven females were given one full injection (700 IU HCG·kg^{-1} body weight) during 1600-1630 on 7 June 1991, and five (nos. 13-17) were injected again 24 hours later (five males). Ovulation occurred 3.8 days before the new moon. Oil globule diameter range was 192-222 µm.

Female no.	Body weight (g)	First injection (IU HCG)	Second injection (IU HCG)	Time to fertilization (hours)	Egg diameter (µm)	Number of eggs (1000s)	Fertilization rate (%)	Hatching rate (%)
				Two full doses				
13	700	500	500	34	766	145	94	100
14	1,100	775	775	34	790	507	99	72
15	1,700	1,200	1,200	34	739	471	99	100
				One full + one half dose				
16	900	625	325	34	771	262	88	74
17	1,100	775	400	36	752	188	98	100
				One full dose				
18	800	560	0	0				
19	1,100	775	0	40			210	0
20	1,900	1325	0	0				

treatments were observed. Mean egg diameter was 764 µm. From 1.57 million eggs (females 13-17), 1.32 million larvae hatched. Only one of the three fish injected once released eggs; they flowed freely at 40 hours but were not clear and could not be fertilized.

At 28°C, squaretail coral trout eggs hatched within 18-20 hours after fertilization. Larval eyes were pigmented at about 48 hours after hatching, and feeding on rotifers began at about 60 hours.

Epinephelus fuscoguttatus

Three female and two male brown-marbled groupers were collected on 5 and 6 June. Initial biopsies were not taken. In trial Ef-1, both females received a full first injection of 700 IU HCG·kg^{-1}; the next day one was given a full dose and the other a half dose. In trial Ef-2, the female was given a single full injection.

Trial Ef-1: By 31 hours after the first injection, biopsied oocytes from females 1 and 2 were clear, but they did not flow easily until 37 hours (Table 4). An additional 42,000 eggs were found in the holding tank.

Table 4. Trials Ef-1 and Ef-2: Induced spawning of *Epinephelus fuscoguttatus*. Females no. 1 and 2 were injected (700 IU HCG·kg^{-1} body weight) at 1400 on 5 June 1991 and 24 hours later (two males). Ovulation occurred 5.7 days before the new moon. Female no. 3 was injected only once at 1530 on 6 June 1991, and ovulation occurred 4.6 days before the new moon. Oil globule diameter range was 201-230 µm.

Female no.	Body weight (g)	First injection (IU HCG)	Second injection (IU HCG)	Time to fertilization (hours)	Egg diameter (µm)	Number of eggs (1000s)	Fertilization rate (%)	Hatching rate (%)
				Two full doses				
1	4,500	3,150	3,150	37	780	1,148	94	32
				One full + one half dose				
2	10,100	7,070	3,500	37	816	2,650	99	94
				One full dose				
3	4,500	3,150	0	39	771	1,237	99	80

Trial Ef-2: Fertilization of eggs from female 3 was attempted three times. Stripping at 38.5 hours after the single injection resulted in 96% fertilization and 52% hatching; at 39 hours, 99% fertilization and 80% hatching (Table 4); and at 39.5 hours, 91% fertilization and 66% hatching.

Mean diameter of brown-marbled grouper eggs was 789 μm. At 28°C, eggs hatched within 18-20 hours after fertilization. From 5.04 million eggs, 3.79 million larvae hatched. Larval eyes were pigmented at about 48 hours after hatching and feeding on rotifers began at about 60 hours.

Low survival in trial Pa-1 might have partly resulted from inexperience with the species and facility. Survival in trial Pa-3 was probably more typical. Numbers of eggs per kg from these two species were higher than for some species that have larger eggs, such as Nassau grouper (920 μm diameter, typically 100,000-230,000 eggs/kg from 2.2-5.3 kg females, Tucker et al. 1991). When ovulation was induced in squaretail coral trout, about twice as many eggs were released as were naturally in trial Pa-2. With good timing, 0.2 million squaretail coral trout or brown-marbled grouper hatchlings could be expected for each kg of female weight. The potential for seed production with these and other groupers is high.

Table 5. Summary of spawning trials with *Plectropomus areolatus* and *Epinephelus fuscoguttatus* in Palau during May and June 1991. Listed are mean number of eggs per female and per kg female weight.

Female no.	Eggs/female (1,000s)	Eggs/kg (1,000s)
Plectropomus areolatus		
1-5	486	258
6-9,11	171	124
13-17	315	286
Epinephelus fuscoguttatus		
1-3	1,678	264

Diameter of preserved eggs from Palau (789 μm) was smaller than for eggs obtained by other researchers from Indonesia and Singapore (890 μm) but similar to those from Saudi Arabia (766 μm). This could represent a real size difference between populations or an artifact resulting from differences in spawning and handling. Kohno et al. (1990b) used increasing doses of 150, 300 and 600 IU HCG·kg^{-1}, 24 hours apart, for both male and female, to induce voluntary spawning of a pair of brown-marbled groupers 62 hours after the first injection, in Indonesia. During 2 days of spawning, the 5.7 kg female produced 4.00 million eggs, of which 1.12 million hatched. Average egg diameter was 890 μm (oil globule 200 μm) Voluntary spawning in 75-m^3 cages in Singapore was reported by Lim et al. (1990). Spawning usually began between the last quarter and new moon. Average egg diameter was 890 μm (range 870-920 μm, oil globule 190-210 μm). Voluntary spawning in 5 to 30-m^3 concrete tanks in Indonesia was reported by Mayunar et al. (1991). Egg diameter range was 852-935 μm (oil globule 198-238 μm). Maneewong and Hazza (1992) had this species spawn in a 25-m^3 round fiberglass tank in Saudi Arabia. Egg diameter was 766 μm (oil globule 195 μm).

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