# Induced Spawning of Nassau Grouper *Epinephelus striatus*

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## Abstract

**Nassau grouper *Epinephelus striatus* females ovulated 48–51 h after the first of two intramuscular injections of human chorionic gonadotropin given 24 h apart (usually 0.7 IU/gram body weight). Typical spawns contained 400,000–600,000 eggs. With fresh milt and clean water, fertilization rate was 85 and 86%. Survival from fertilization to first feeding for six spawns was 73–94%.**

The Nassau grouper (*Epinephelus striatus*, family Serranidae) ranges from Bermuda and South Carolina to Brazil, including the Caribbean Sea and Gulf of Mexico. Because it is a popular food fish, numerous stocks have been depleted and the Nassau grouper is considered a high-ranking candidate for aquaculture. Spawning aggregations have been reported to occur during the winter at the eastern ends of islands and offshore banks (e.g., Bahamas, Bermuda, Bimini, Cayman Islands, Cuba, Virgin Islands) and along eastern projections and atolls of the Belizean barrier reef. Running ripe males and ripe females usually can be caught from spawning aggregations.

Although not suitable for all species tested, human chorionic gonadotropin (HCG) has been used, alone and combined with other substances, to induce ovulation in a variety of fish (Lam 1982; Zohar 1989). During the 1987, 1988, and 1989 spawning seasons in Grand Cayman, HCG was used to induce ovulation in Nassau groupers, and the larvae were reared beyond first feeding (Tucker, in press). The techniques can be used to produce hundreds of thousands of eggs per spawn for aquacultural purposes.

## Methods

Adult male and female groupers were caught by hook and line fishing at (1) 20–30 m depth at Coxswain Bank at the northeast corner of Grand Cayman's fringing reef system, and at (2) 35 m depth south of Sand Cay off the southwest corner of Grand Cayman (Fig. 1). Capture occurred in the morning during 0800–1200 local standard time (the same as U.S. eastern standard time, EST). Fish were transported to the laboratory and held in 1,000 or 2,000 liter fiberglass tanks in running ambient seawater (34–37 ‰ salinity, 26–28 C) near the Cayman Turtle Farm (Fig. 1). A 22 gauge hypodermic needle was inserted into the abdominal cavity of each fish to vent excess gas from the burst gas bladder.

Males were running ripe and did not require injections, but two were given single intramuscular injections of 0.35 IU/gbw (grams body weight) HCG as a trial. Females were injected with HCG during the afternoon within 3–7 h after capture and again 24 h later if ovulation had not occurred. After ovulation, clear spherical eggs flowed freely from the female when gentle pressure was applied to the central abdomen. Overmature eggs generally had lost their transparency, had begun to collapse and to lose their spherical shape, and had obvious structural irregularities.

Fertilization, incubation, and rearing were done in natural seawater (cartridge-filtered to 5 $\mu$m, nominal pore size). Milt was col-

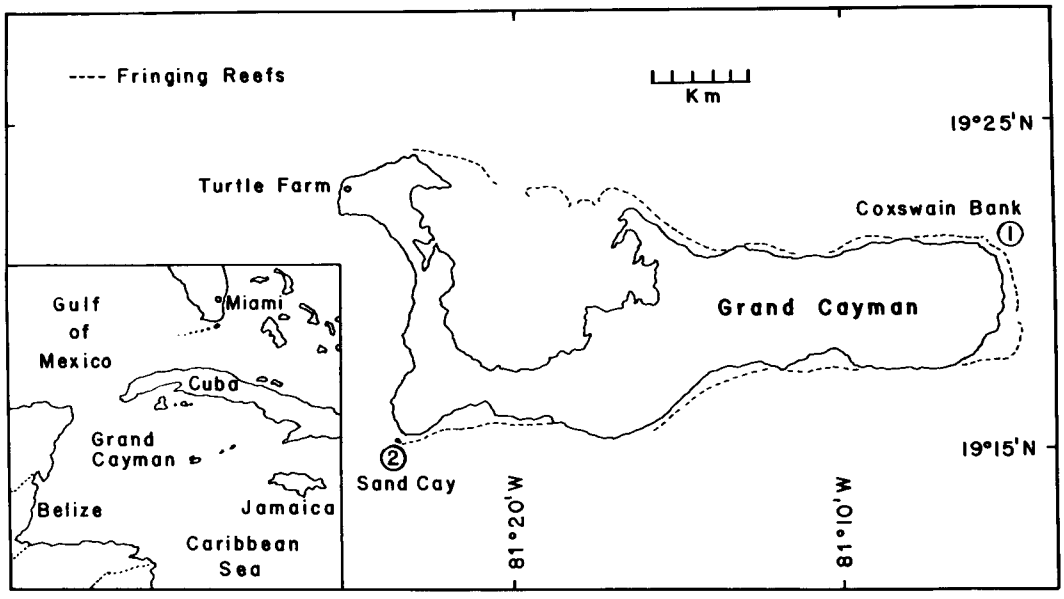


FIGURE 1. *Map of Grand Cayman, showing sources of Nassau grouper broodstock at Coxswain Bank (1) and Sand Cay (2). Fish were maintained and spawned near the Turtle Farm.*

lected in hypodermic syringes (without needles) prior to stripping eggs. After ovulation was detected, eggs were stripped from the female into a plastic beaker containing 300 ml seawater. Milt was added immediately and the mixture was swirled. After 5 min, milt and debris were washed away with clean water. Total egg number was estimated volumetrically (1,200 eggs/ml). A sample of 50–100 fertilized eggs from each spawn was placed in 2 or 4 liter beakers of seawater for monitoring survival; eggs and larvae were kept at 25–28 C until more than 48 h after hatching. Larger numbers of larvae were reared in 30, 1,000, and 2,000 liter tanks.

## Results and Discussion

Ovarian development and spawning in this species are highly synchronized. Spawning of Nassau groupers near the Cayman Islands typically occurs over a short period at or just after the full moon in January and February; spawning might also occur near the time of full moon when it is in late December or early March. During a concurrent study (Tucker and Bush, unpublished), the minimum size of both females with yolked eggs and males with running milt was 2 kg, 50 cm total length. *Epinephelus tauvina* and *E. amblycephalus* mature at about the same size, when they are 2–3 years old (Chen et al. 1977; Tseng and Poon 1983). During 1989, the authors of the present study examined gonads from 263 Nassau groupers, including 170 mature females. Natural hydration of ovaries (absorption of water by and clearing of oocytes) began just after the full moon in January and February, and spawning occurred a few days later; within 8–10 d after the full moon, spawning was finished and the aggregations had dispersed. For several days before hydration begins, nearly every mature female in the aggregation is ripe (with fully yolked oocytes, full yellow color with well developed ovarian blood vessels) and is susceptible to HCG induced ovulation. Ripe females also have a characteristic external bulge between the anus and the urinary papilla. Ovulation can be induced later in the cycle during hydration and before natural ovulation, but not

TABLE 1. *Induced spawning of Nassau grouper* (Epinephelus striatus). *Daily water temperature range was 26–28 C. CB = Coxswain Bank; SC = Sand Cay. Number of days from full moon to capture time is indicated. EST = U.S. eastern standard time = local standard time.*

| Female no. | Fish from | Date of first injection | Full moon (d) | Time of injection (EST) | Female weight (g) | First injection (IU) | Second injection (IU) | Time to fert. (h) | Number of eggs (1,000s) | Fert. (%) | Fert. to hatch (%) | Hatch to 24 hours (%) | 24 to 48 hours (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1987 | | | | | | | | | | | | | |
| 1 | CB | 14 Jan | 0 | 1700 | 2,900 | 2,600 | 2,200 | 50 | 400–600 | Low[a] | High | High | High |
| 2 | CB | 15 Jan | +1 | 1400 | 4,650 | 2,800 | 2,200 | 101 | 100–200 | 0[bc] | | | |
| 3 | CB | 16 Jan | +2 | 1200 | 2,200 | 2,200 | 2,200 | 48 | 400–600 | 32[d] | 96 | 100 | 98 |
| 4 | CB | 16 Jan | +2 | 1200 | 3,200 | 2,200 | 2,200 | 50 | 548 | 86[c] | 94 | 85 | 98 |
| 1988 | | | | | | | | | | | | | |
| 5 | CB | 6 Feb | +4 | 1230 | 4,750 | 3,325 | none | 29 | 630 | 85[c] | 95 | 82 | 99 |
| 6 | SC | 8 Feb | +6 | 1400 | 4,300 | 3,010 | 2,600 | | 0 | | | | |
| 7 | SC | 8 Feb | +6 | 1600 | 2,350 | 1,650 | 1,650 | | 0 | | | | |
| 1989 | | | | | | | | | | | | | |
| 8 | CB | 20 Jan | -1 | 1200 | 4,425 | 3,100 | 3,100 | 51 | 500–600 | 56[ce] | 79 | 92 | 100 |
| 9 | SC | 20 Feb | 0 | 1200 | 5,275 | 3,700 | 3,700 | 53 | 400–600 | 0[cef] | | | |
| 10 | CB | 20 Feb | 0 | 1330 | 4,500 | 3,150 | 3,150 | 52 | 400–600 | 0[cef] | | | |
| 11 | CB | 20 Feb | 0 | 1330 | 3,050 | 2,125 | 2,125 | 49 | 13 | 1[ce] | 86 | 94 | 97 |
| 12 | SC | 27 Feb | +7 | 1440 | 8,625 | 6,050 | 6,050 | 50 | 1 | 15[ce] | 84 | 96 | 98 |

[a] Used 9 h old refrigerated milt from dead male.
[b] Overmature eggs.
[c] Used milt from live male.
[d] Used 1 ml milt from live male and 2 ml milt from dead male.
[e] Toxic levels of ammonia in seawater system.
[f] Aborted eggs.

as reliably because of apparent disruption of the natural process. Immediately before spawning (probably late afternoon), running ripe females might be caught, but observations in this study indicate that the availability interval for ovulated eggs is probably very short (maybe a few hours), and groupers appear to be more difficult to catch when close to spawning. Soon after spawning, some of the oocytes remaining in ovaries can still be ovulated in some fish. Taking ovarian biopsies from Nassau groupers without causing injury and infection is very difficult because the oviducts do not have an external opening until just before spawning. Synchronization of the natural spawning cycle permitted a high degree of confidence in predicting when females were susceptible to induced ovulation.

Seven of the 12 females produced viable eggs from which larvae were reared (Table 1). Dosage was typically 0.7 IU/gbw (range 0.5–1.0). Females 1, 3, 4, 8, and 11 were caught when hydration was beginning; ovulation occurred 48–51 h after the first injection of HCG. Female 5 was caught 4 d after the full moon, between the beginning of hydration and spawning; ovulation occurred 29 h after a single injection. Ovulation was detected too late in female 2 (overmature eggs), which had received low doses of HCG and might have been slow to respond. In some rearing trials, mortality of larvae was tentatively linked to ammonia sometimes present in the seawater supply. Low, but possibly toxic, levels of hydrogen sulfide might also have been present when ammonia was detected. Apparently, females 8–12 were stressed by toxic levels of ammonia (up to 1.5 mg/L total ammonia nitrogen (TAN)) or other factors from the seawater system, which had been damaged

by a hurricane in late 1988. Eggs from females 9 and 10 were not viable. Female 11 produced a small number of eggs, which did not fertilize well. Female 12 was caught near the end of the February spawning period and produced only 1,100 eggs, which also did not fertilize well. Water quality during 1989 not only stressed adults but apparently also reduced fertilization rates.

Eggs were 0.9 mm in diameter and were neutrally buoyant in water of 32 ‰ salinity (normal salinity near the Cayman Islands is 35–37 ‰). Hatching occurred within 27–29 h after fertilization at 25 C and within 23–25 h at 28 C. Larvae had pigmented eyes 48 h after hatching and began feeding within 60 h.

HCG has been used to induce ovulation in other *Epinephelus* species. Chen et al. (1977) induced ovulation and spawning in *E. tauvina* with two to four injections of HCG (average 0.6 IU/gbw, range 0.5–0.8) and one or two injections of snapper or salmon pituitary extract. Ovulation occurred 1.5–5 d after the first injection. One fish ovulated 69 h after a single injection of HCG. Twelve females ovulated completely. Fertilization rates were only 30–40%, but hatching rates were high. Tang et al. (1979) used two (1.0 IU/gbw) and three (0.7 IU/gbw) injections of HCG plus 500 IU pregnant mare serum/fish to induce ovulation in two *E. amblycephalus* 6 and 5 d after the first injection. Fertilization rates were 10% and 35%, with hatching rates of 5% and 11%. Tseng and Poon (1983) induced ovulation in *E. amblycephalus* with one to three injections of HCG (usually 1,000 IU per injection) and fertilized the eggs with milt from *E. akaara*. Fertilization rates were 30–90% and hatching rates about 50%. Tseng and Ho (1979) used two injections of HCG (2 IU/gbw, except second dose to one was 1 IU/gbw) to induce ovulation in four *E. akaara* 38–67 h after the first injection. One spawn had a 98% fertilization rate and 75% hatching rate. In the present study, fertilization rate was 85% and 86% when fresh milt and clean water were used. Hatching rate for six spawns was 79–96%, and survival from hatching to first feeding was 81–98%.

One day after receiving HCG injections, the two males produced more milt than would be expected from untreated fish. If milt is in short supply, injecting males would probably increase the short term supply of milt; however, injected male fish usually regress and stop producing milt more quickly.

Because Nassau groupers have well-defined spawning locations and times, induced ovulation can be a reliable method of obtaining large numbers of eggs. However, ripe broodstock are available for only several weeks, and because aggregation sites are in some of the most exposed reef areas, weather can hinder collection efforts.

Female groupers caught from spawning aggregations from several days before the full moon until natural ovulation occurs are likely to ovulate after one or two injections of 0.7 IU HCG/gbw. Before hydration occurs, two injections are needed and time to ovulation is expected to be 48–51 h. After natural hydration begins, one injection might be enough and the time should be shorter.

At least four species have spawned voluntarily in captivity: *E. akaara* in an 8 cubic meter pond (Ukawa et al. 1966); *E. striatus* in a public aquarium (Manday and Fernandez 1966); *E. andersoni* in a public aquarium (Ballard 1971); *E. tauvina* in 90 and 400 cubic meter concrete tanks (Hussain et al. 1975; Hussain and Higuchi 1980; Abdullah et al. 1984). Larvae of *E. striatus* and *E. andersoni* were not reared. With large tanks and good water, nutrition, and management, Nassau groupers probably could be conditioned to spawn voluntarily.

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## Literature Cited

**Abdullah, M. A. S., S. Akatsu, K. Al-Abdul-Elah and S. K. Teng.** 1984. Refinement of spawning and larval rearing techniques in hamoor (*Epinephelus tauvina*). Kuwait Institute for Scientific Research, Annual Research Report (1983):55–57.

**Ballard, J.** 1971. Aquarium briefs—mating in captivity. Bulletin of the South African Association Marine Biology Research (9):31–33.

**Chen, F. Y., M. Chow, T. M. Chao and R. Lim.** 1977. Artificial spawning and larval rearing of the grouper, *Epinephelus tauvina* (Forskal) in Singapore. Singapore Journal of Primary Industries 5:1–21.

**Hussain, N. A. and M. Higuchi.** 1980. Larval rearing and development of the brown spotted grouper, *Epinephelus tauvina* (Forskal). Aquaculture 19: 339–350.

**Hussain, N., M. Saif and M. Ukawa.** 1975. On the culture of *Epinephelus tauvina* (Forskal). Kuwait Institute for Scientific Research, State of Kuwait.

**Lam, T. J.** 1982. Applications of endocrinology to fish culture. Canadian Journal of Fisheries and Aquatic Sciences 39:111–137.

**Manday, D. G. and M. J. Fernandez.** 1966. Desarrollo embrionario y primeros estadios larvales de la cherna criolla, *Epinephelus striatus* (Bloch) (Perciformes: Serranidae). Acadamia de Ciencias de Cuba, Instituto de Oceanologia Estudios, Havana 1:35–45.

**Tang, H. C., J. Y. Twu and W. C. Su.** 1979. Preliminary report on artificial propagation of blackspotted grouper, *Epinephelus amblycephalus* Bleeker. Bulletin Taiwan Fisheries Research Institute 31:511–517.

**Tseng, W. Y. and S. K. Ho.** 1979. Egg development and early larval rearing of red grouper (*Epinephelus akaara*, Temminck & Schlegel). Quarterly Journal Taiwan Museum 32:209–219.

**Tseng, W. Y. and C. T. Poon.** 1983. Hybridization of *Epinephelus* species. Aquaculture 34:177–182.

**Tucker, J. W., Jr.** In press. Growth and development of domestic juvenile Nassau groupers. Symposium on the biology and management of Caribbean groupers. Proceedings of the 43rd Gulf and Caribbean Fisheries Institute Conference (1990).

**Ukawa, M., M. Higuchi and S. Mito.** 1966. Spawning habits and early life history of a serranid fish, *Epinephelus akaara* (Temminck et Schlegel). Japanese Journal of Ichthyology 13:156–161.

**Zohar, Y.** 1989. Fish reproduction: its physiology and artificial manipulation. Pages 65–119 *in* M. Shilo and S. Sarig, editors. Fish culture in warmwater systems: problems and trends. CRC Press, Boca Raton, Florida, USA.