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Hormone-Induced Multiple Spawning of Captive Nassau Grouper Broodstock

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Abstract.—During a 5-month period from 3 December 1991 to 28 April 1992, two female Nassau grouper *Epinephelus striatus* were spawned three times and one female two times by hormone induction. The interval between spawnings for an individual female ranged from 28–75 d. Successful spawnings (fertilization rate > 50%) were induced in females with average oocyte diameters ranging from 517–544 μm by a primary injection of human chorionic gonadotropin (HCG; 1,000 IU/kg body weight), followed by a second injection (500 IU/kg body weight) after 24 h. Time of strip-spawning ranged from 37–45 h after the first injection and was inversely related to average water temperature (range, 22.4–26.4°C). Mean diameters spawned eggs were 871–959 μm, and the number of eggs spawned per female ranged from 200,000 to 2,000,000. Fertilization and hatching success were 17.9–80.6% and 68.3–90.1%, respectively. The results demonstrate a reproductive capacity that minimizes broodstock requirements and enhances suitability of the Nassau grouper for aquaculture.

The Nassau grouper *Epinephelus striatus* is an important food fish in the Caribbean and tropical western Atlantic regions (Sadovy, in press) and is the most important commercial finfish in the Bahamas (Colin 1992). This species forms large spawning aggregations during full-moon periods of 1 or 2 months in winter in the Bahamas and mid-Caribbean regions (Smith 1972; Colin 1992; Tucker et al. 1993). Overfishing (Smith 1972; Olsen and La Place 1978; Colin et al. 1987; Carter 1988; Carter et al. 1994) has resulted in significant declines in landings in recent years off Puerto Rico, the U.S. Virgin Islands, Belize, Cuba, Dominican Republic, Jamaica (Munro 1983; Bohnsack 1989; Sadovy 1993 and in press), and Bermuda (Bannerot et al. 1987). In the United States, the Nassau grouper is a candidate for the endangered species list (Sadovy, in press).

Tucker et al. (1991) and Colin (1992) reported successful induced spawning of ripe Nassau grouper captured from spawning aggregations by injecting fish with human chorionic gonadotropin (HCG). Since capture of ripe spawners is difficult and possible for only brief periods each year (Tucker et al. 1991, 1993; Colin 1992), the development of husbandry methods which allow greater control of maturation and spawning is important for aquaculture. In this paper, we report results of preliminary experiments on induced spawning of a captive Nassau grouper broodstock.

Five male and three female adult Nassau grouper (3.2–10.4 kg, 46–65 cm standard length) were trapped off Lee Stocking Island, Exuma Cays, Bahamas (23°45′N, 76°10′W) at depths of 18–30 m between August and December 1991. Fish were maintained in a 15-m^3, circular (diameter, 4.57 m; depth, 0.92 m) fiberglass tank supplied with flow-through (87 L/min) seawater (36–38‰), aeration, and an 80% light-occluding cloth cover for shade. Fish were fed a diet of frozen squid, fish, and a commercial grower feed for striped bass *Morone saxatilis* that contained 38% protein (Zeigler Brothers, Inc. Gardners, Pennsylvania).

Gonadal maturity in females was monitored at approximately 4-week intervals by biopsy with a polyethylene cannula (1.52 mm outer diameter, 0.86 mm inner diameter) inserted through the genital pore. Stage of oocyte maturity was determined by measuring diameters (Shehadeh et al. 1973) under a microscope fitted with an ocular micrometer. The presence of milt in males was checked by applying pressure to the abdomen.

Induced spawning was attempted in females with mature (postvitellogenic stage) oocytes (Kuo et al. 1974a, 1988). In the first three spawning trials, females were given a single intramuscular injection of human chorionic gonadotropin (HCG; Crescent Research Chemicals, Phoenix, Arizona) at a dose of 1,000 IU/kg body weight. In subsequent trials, females were given a second injection at a dose of 500 IU/kg body weight 24 h after the first injection. Males released copious amounts of milt and were not rejected with hormone. The proper time to strip-spawn females was determined by observing abdominal distention, protrusion of the cloacal region, and a change from a normal color pattern to a "bicolor" phase, characterized

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TABLE 1.—Summary of induced multiple spawning of Nassau grouper broodstock, captured at Lee Stocking Island, Bahamas. Females from successful spawns were injected with human chorionic gonadotrophin (HCG) in one (1,000 IU/kg body weight) or two (1,000 IU/kg body weight followed 24 h later with 500 IU/kg body weight) doses.

Date of first HCG injection	Female	Body weight (kg)	Initial egg diameter (μm)	Time of first injection[a]	Number of HCG injections	Time of stripping[b]	Diameter of spawned eggs (μm)
3 Dec 91	1[e]	6.8	519	1,200	1	40.0	940
10 Dec 91	2[e]	3.7	530	1,430	1	42.0	906
26 Dec 91	3	3.5	546	1,600	1	48.0	937
1 Jan 92	1	7.2	538	2,000	2	42.5	925
23 Feb 92	2[e]	3.9	540	1,700	2	39.0	929
3 Mar 92	1	7.4	544	2,000	2	45.0	923
10 Mar 92	3	3.7	517	2,000	2	37.0	959
13 Apr 92	3	3.7	474	2,000	3	No spawn	
28 Apr 92	2	4.0	525	1,930	2	43.5	871
4 May 92	1	7.6	484	1,800	3	No spawn	

[a] Hours Eastern Standard Time.
[b] Hours after first injection.
[c] Percent of larvae hatched from fertilized eggs.
[d] Negative symbol = days before full moon; positive symbol = days after full moon.
[e] Female released some eggs prior to strip spawning.
[f] Estimated from relationship between fertilization rate (y) and percentage buoyant eggs (x): $y = 1.2947x - 51.8578$ ($r^2 = 0.8921$; $N = 4$; $P < 0.001$).

by a dark upper body and a white lower body, as previously reported for Nassau grouper in natural aggregations (Smith 1972; Colin 1992; Tucker et al. 1993). A third injection of HCG at 500 IU/kg body weight was given if eggs could not be stripped by 48 h after the first injection.

Eggs were fertilized artificially by the wet method. Approximately 8–10 mL of milt from two or three males was added to 11 L of seawater and mixed. Eggs were stripped from the female into a dry 8-L bucket, added to the milt–seawater mixture, stirred, allowed to water-harden for approximately 10 min, then rinsed with seawater. Diameters of fertilized eggs ($N \geq 100$) were measured.

Total number of eggs released per spawn was determined volumetrically. Fertilization rate was expressed as the percentage of developing eggs (Kuo et al. 1973) or estimated from the percentage of buoyant eggs (Table 1). Hatching rates were determined as the percentage of larvae hatched from five replicate samples of fertilized eggs ($N \geq 100$ eggs/sample) incubated in 4-L beakers containing 1.2 μm filtered seawater (36–38‰) supplied with gentle aeration. Eggs were maintained at a temperature of 25.0 ± 0.3°C (mean ± SE) under fluorescent lighting at a photoperiod of 11 h light and 13 h darkness.

During a 5-month period, from 3 December 1991 through 28 April 1992, eight spawnings were obtained from three females (Table 1). Females 1 and 2 were spawned three times, and female 3 was spawned two times. The interval between spawnings for an individual female ranged from 28 to 75 d (Table 1).

Available information suggests that spawning of Nassau grouper in nature is restricted to the full-moon periods during the winter months of January and February in the mid-Caribbean region (Tucker et al. 1993), December and January in the southern Bahamas (Colin 1992; Tucker et al. 1993), and January and February in the northern Bahamas (Tucker et al. 1993). Results of the present study with captive Nassau grouper reveal a potential for sequential spawning over an extended period in nature and have important implications regarding origin of larvae and juveniles observed in the field.

Spawning of Nassau grouper was induced with one (1,000 IU/kg body weight) or two (1,000 IU/kg, then 500 IU/kg body weight) injections of HCG when mean initial oocyte diameters were 517–546 μm, but fertilization success appeared more variable with a single injection (Table 1). Similar results were obtained by Kuo et al. (1988) with the bluespotted grouper *E. fario*.[2] Tucker et al. (1991) used primarily two injections of HCG at doses that averaged 700 IU/kg body weight to induce spawning in ripe female Nassau grouper captured from a spawning aggregation. Successful HCG-induced spawning of other *Epinephelus* spe-

[2] The American Fisheries Society prefers the name *E. argus* for this species.

TABLE 1.—Extended.

Date of first HCG injection	Number of eggs (1,000s)	Bouyant eggs (%)	Fertilization rate (%)	Hatch rate[c] (%)	Days to full moon[d]	Last spawn (d)	Bicolor before spawn
3 Dec 91	1,000	95.0	80.6[f]		+12		yes
10 Dec 91	250	50.0	17.9[f]		−11		no
26 Dec 91	200	50.0	17.9[f]		+5		no
1 Jan 92	2,000	90.0	73.7[f]		+11	28	yes
23 Feb 92	288	85.4	62.2	71.4	+5	75	yes
3 Mar 92	1,860	88.7	72.4	88.3	+13	62	yes
10 Mar 92	220	92.9	80.1	90.1	−8	75	yes
13 Apr 92					−5	30	
28 Apr 92	1,326	76.1	56.4	68.3	+11	65	yes
4 May 92					−12	62	

cies has been obtained with two injections at doses of 500–1,000 IU/kg body weight (*E. amblycephalus,* Tseng and Poon 1983; *E. salmonoides,* Huang et al. 1986; *E. akaara,* Tseng and Ho 1988).

The frequency distribution of oocyte diameters of females that were successfully strip-spawned (i.e., fertilization rate > 50%) was unimodal (Figure 1, a–c), with mean oocyte diameters at first injection ranging from 517 to 546 μm (Table 1). No spawnings were obtained from females with initial oocyte diameters of 474 μm and 484 μm (Table 1), suggesting that oocytes were below a minimum size necessary for HCG-induced maturation. Frequency distributions of oocyte diameter became more dispersed during the nonspawning summer period (Figure 1, d–e). Males stopped producing milt by July, and eggs could not be obtained from female 3 by August and from female 2 by September.

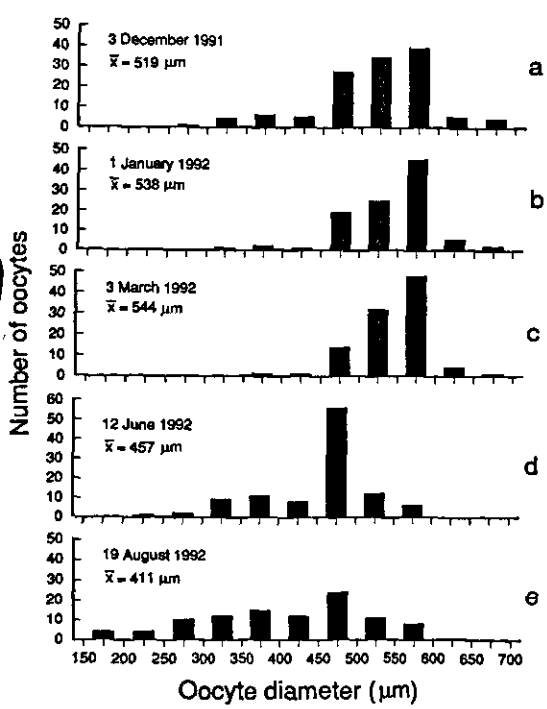

FIGURE 1.—Frequency distribution of oocyte diameters from a Nassau grouper (female 1). Samples were taken (a–c) prior to successful HCG-induced spawning from December to March and (d, e) during the nonspawning period from June to August.

For successful spawnings, time to stripping ranged from 37 to 45 h after first injection and generally decreased with increasing water temperature. Premature strip-spawning or a delay of a few hours can lower spawning and fertilization success (Shelton 1989; Tucker et al. 1991; Battaglene and Talbot 1992). Additional data is needed to establish the relationships between water temperature and the rate of oocyte growth during final maturation (Shelton 1989) in Nassau grouper as a basis for predicting optimum time of strip spawning.

The mean diameter of spawned, water-hardened eggs ranged from 871 to 959 μm and the number of eggs spawned per female ranged from 200,000 to 2,000,000 (54,045–340,000 eggs/kg body weight). Hatching rate ranged from 68.3–90.1% (Table 1).

During the 5-month spawning period (3 December 1991 to 28 April 1992), mean daily water temperatures ranged from 22.4–26.4°C (mean, 24.5°C), and day length (hours of daylight) ranged from 10.9 h to 12.8 h (Figure 2a). Average oocyte diameters among females exceeded 500 μm from December to March (Figure 2b), and water temperatures and day length were near annual minima. Oocyte di-

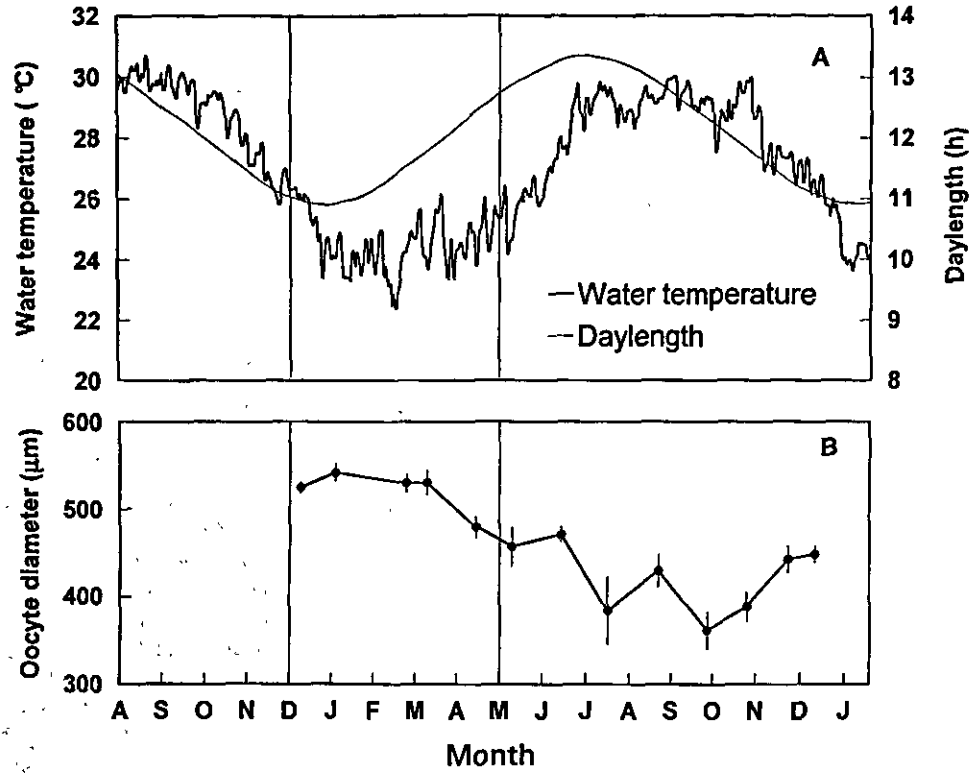

FIGURE 2.—(A) Mean daily water temperature in Nassau grouper broodfish tanks and day length (hours of daylight) at Lee Stocking Island, Bahamas, from August 1991 to December 1992. The induced spawning period (3 December 1991 to 28 April 1992) is bounded by vertical lines. (B) mean oocyte diameters of captive female broodstock over a 12-month period. Plotted points represent means (±SE) for three females, except for the months of August (two females) and September (one female) when oocytes could not be obtained from some individuals. One hundred or more oocytes from each female were measured, except during the months of September–November, when sample sizes ranged from 20–103 oocytes/female.

ameters began to decrease in April (Figure 2b), as water temperature and day length increased (Figure 2a). Oocyte diameters reached minimum levels during July–October, when water temperature and day length were near annual maxima. An increase in oocyte diameters in November–December 1992 (Figure 2b) was associated with falling water temperatures and near minimum day length (Figure 2a). The correlative data provide a basis for developing artificial photothermal regimes for inducing out-of-season maturation, as has been demonstrated in other winter-spawning species, including striped mullet *Mugil cephalus*, sea bass *Dicentrarchus labrax*[3] and gilthead bream or seabream *Sparus aurata* (Kuo et al. 1974b; Zohar 1989).

Induced-spawning success was unrelated to moon phase (Table 1). However, the presence of spawned eggs in the brood tank demonstrated that natural spawning in captivity without hormone induction occurred on the evening of 23 December, 2 d after the full moon, but with low fertilization success (<5%). This suggests that natural, voluntary spawning of Nassau grouper in captivity is possible, as was previously observed by other workers (see Tucker 1994 for review). Natural spawnings would reduce handling stress and may promote fertilization success and egg quality.

To summarize, Nassau grouper broodstock were maintained in captivity for extended durations, and females were induced to spawn by HCG injection two or three times over a 5-month reproductive season with no adverse effects on fish health. These findings reveal a hardiness and reproductive potential that minimize broodstock requirements

[3] The American Fisheries Society prefers to refer to this species as European bass *Morone labrax*.

and enhance the potential of this species for commercial aquaculture.

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