Voluntary Spawning of Captive Nassau Groupers *Epinephelus striatus* in a Concrete Raceway

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Abstract

Nassau groupers *Epinephelus striatus* held in a 37-m³ concrete raceway were conditioned to spawn 3 mo later than in their home waters. After being held in the raceway for 15 mo, four female and two male groupers spawned voluntarily over a 4-d period in March 1994, producing 10.3 million eggs. By December 1994, two females had been removed. During March 1995, one of the remaining two females spawned on three consecutive days (60 cm TL, 1,172,000 eggs) and the other only on the third day (47 cm TL, 488,000 eggs). In April 1995, three females (including one held in isolation for 6 mo, then replaced 6 d before the first spawn) produced more than 9.8 million eggs in 4 d. The isolated female spawned 8 d after being placed in a cage in the raceway and 4 d after release in the raceway.

Individual females spawned as many as nine times a day for 1–4 d. Spawning occurred from 3.5 d before until 8.5 d after the full moon. A 30-d interval elapsed between March and April 1995 spawning periods. Fertilization was in the range 83–100% and hatching 90–100%. Spawns occurred in a temperature range of 23.1–27.9 C; however, based on spawning frequency and volume and on egg development, 24–27 C seems most suitable. These results and other evidence support the view that Nassau groupers can be conditioned to spawn any month of the year, mainly by manipulating temperature.

The Nassau grouper *Epinephelus striatus*, family Serranidae, is a marine reef fish of the greater Caribbean region well-known for its highly synchronized spawning cycle (Colin 1992; Tucker et al. 1993; Carter et al. 1994). From dozens to many thousands of individuals aggregate at traditional sites to spawn. Spawning at each site usually occurs during 2–3 mo, with peak activity near the full moon.

Three previous references have reported observation of spawning by Nassau groupers. At about 2200 h on 22 April 1963, several spawned in the National Aquarium in Havana, Cuba (Guitart and Juarez 1966). This was 13 d after the full moon and 1 d before the new moon, but lighting was not entirely natural. Water temperature was 24.9 C. Description of spawning was minimal, and no estimate of egg number was given. Two recent articles (Colin 1992; Tucker et al. 1993) described and illustrated spawning events, with both males and females assuming a bicolored pattern (upper body very dark and lower body nearly white). Colin (1992) observed natural spawning in the Bahamas during 20 min before until 20 min after sunset at 21–35 m depth. Spawning occurred from 1 d before until 3 d after the full moon in December 1988 and from 2 d before until 2 d after the full moon in January 1989. Captive Nassau groupers spawned during a Cayman Islands Natural Resources Laboratory study conducted to validate aging of groupers by counting otolith rings. Four females and one male were caught during May–July 1990, injected with tetracycline, and held in a 26-m³ cage on a 9-m deep natural reef off Grand Cayman (Tucker et al. 1993). During 1900–2000 h (about 1 h after sunset) on both 6 and 8 February 1991 (8 and 10 d after full moon, last quarter on 6 February), the fish spawned. Each time, divers observed two females quickly swim horizontally and release eggs, with the male fol-

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lowing them. None of these reports were directly related to aquaculture programs. Spawning in the aquarium and cage were unexpected.

As one phase of a long-range program to develop aquaculture technology for this species, we have maintained a tank of wild broodstock. This paper describes voluntary spawning of those fish after 15, 27, and 28 mo in captivity.

Materials and Methods

On 8 December 1992, two male (4.8, 5.6 kg) and four female (3.6, 3.9, 4.1, 4.8 kg) wild Nassau groupers were transported from the Bahamas to our laboratory near Fort Pierce, Florida, USA. The males were running ripe and the females had just ovulated naturally. They were placed in an outdoor raceway. By December 1994, two of the females had been removed from the raceway, leaving two females and two males. Because of an eye injury, one of the removed females was held alone for six mo (beginning 17 October 1994) in a 300-L cylindrical fiberglass tank under a greenhouse roof identical to the one over the spawning tanks; water temperature fluctuated to a greater extent than in the raceway, but average temperature was similar. On 14 April 1995, the isolated fish was placed in a 1.8 × 1.8 × 1.0 m-deep, 3-mm mesh nylon cage in the raceway to begin social acclimation, and just before sunset on 18 April, she was released into the raceway with the other four groupers.

The raceway, containing 37 m^3 of water (12.2 m × 3.4 m × 0.9 m deep), was under a translucent fiberglass roof, oriented lengthwise from north to south. During March and April, transmission of ambient natural light ranged from 27–37% at noon (rising from 110,000 lux on 21 March to 125,000 lux on 22 May outside with clear sky) to 25–28% at sunset. To prevent overheating of water during late spring to fall, shadecloth was placed over the roof, reducing midday light transmission to 8%. Water from the Indian River estuary was continuously supplied (12 L/min) to the raceways after passing through a slow gravel filter, two rapid sand filters, and 35-μm polyester cartridge filters. Raceway water was continuously recirculated (48 L/min) through separate rapid sand filters and fluidized-bed sand filters. Water temperature was near ambient.

During 1992–1994, the groupers were fed once a day in the late afternoon approximately equal amounts (by dry weight) of: 1) a pelleted dry broodfish diet developed at Harbor Branch (HB9261, 58.6% protein, 11.8% fat, dry-basis); and 2) freshly frozen seafood, on alternate days. The seafood was frozen for at least 48 h to minimize parasite transmission and consisted mostly of scraps of mackerel, salmon, swordfish, and dolphin, plus cleaned squid, with smaller amounts of shrimp tails, cleaned conch, whole menhaden and mullet, and scraps of tilefish, cobia, grouper, snapper, and tuna. During the 12 d before spawning in 1994, the groupers showed a preference for more oily fish. Therefore, salmon was provided for each of the 8 d just before spawning. At other times, they would not eat salmon on consecutive days. During 1995–1996, the diet was similar except that usually pellets were given on Tuesday, Thursday, Saturday, and Sunday, and frozen food on the other three days. In 1995, the fish ate well until 2 d before spawning started in March and until 11 d before the April spawns.

In 1994, videotapes of the fish were made during the spawning period of the first three afternoons (90, 30, and 30 min). In 1995, gamete release usually was clearly visible, and the times are reported. During each session of both years, small egg samples were taken to verify spawning times. Immediately after spawning ceased, egg number was calculated from egg density in ten 100 to 300-mL samples taken from one end of the raceway to the other (eggs were well-mixed in the tank by heavy aeration). Fertilization rate was based on a sample of 100 eggs, at 2–6 h after fertilization. Hatch-

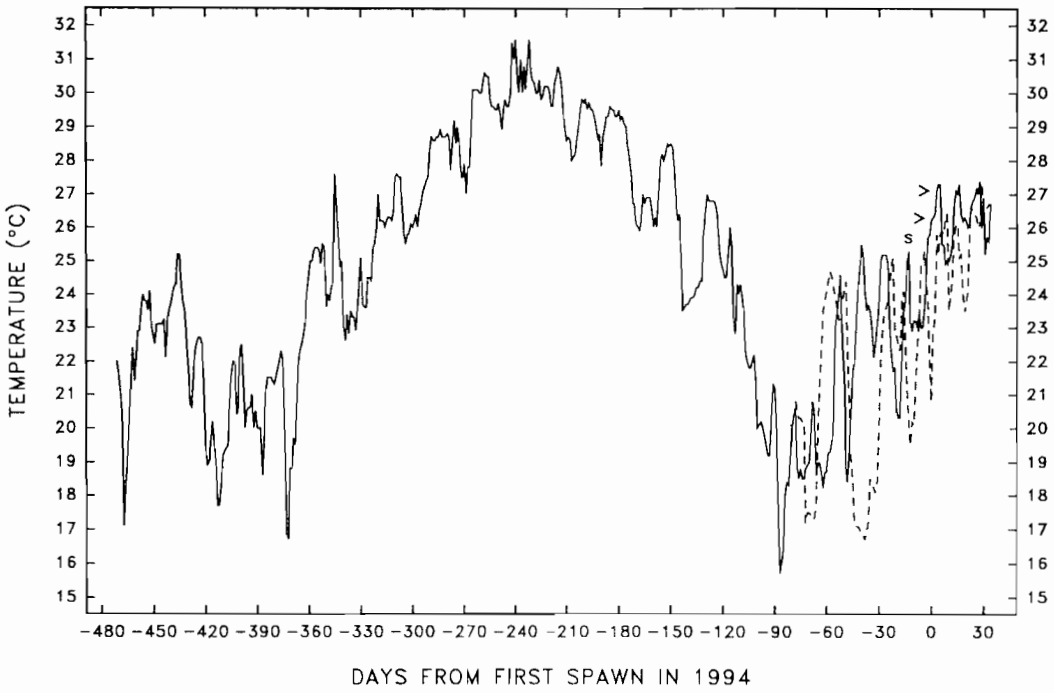

FIGURE 1. *Water temperature for captive wild Nassau grouper broodfish (4 ♀, 2 ♂) in a concrete raceway from 8 December 1992 to 26 April 1994 (solid line). Two females became noticeably swollen at the temperature peak below the "s" (13 March). The ">" symbols indicate first and last spawning days (23–26 March 1994). The dashed line indicates water temperature during 1 January to 26 April 1996.*

ing rate was determined with 100 fertilized eggs held in a 500-mL beaker of filtered water at 26 C and 31–32 parts per thousand (ppt) salinity.

On 1 June 1995, when it was certain the groupers would not spawn again that season, they were measured and weighed. The males were 76 cm TL, 8.4 kg and 79 cm, 9.9 kg. The females not removed were 62 cm, 4.5 kg and 72 cm, 7.5 kg. The removed and replaced female was 66 cm, 5.8 kg.

Results and Discussion

Initial Observations

Males were running ripe during the first week of April 1993, when water temperature was 25–26 C. However, no fish were seen in the bicolored phase, and the females, which had ovulated in December 1992, did not have obvious abdominal swelling until March 1994.

In the fall of 1993, water temperature dropped rapidly through the range for spawning, but during late winter 1994, it rose slowly (Fig. 1). Temperature decreased from 26 C to 23 C during 20 November to 5 December 1993 (15 d), then stayed well below 23 C for 54 d. The annual minimum was 15.7 C on 26 December, 87 d before the first spawn. During 28 January to 23 March 1994 (54 d), temperature rose from 23 C to 26 C. The fish ate well only three times during 26 December–6 February (15.7–24.6 C), then ate well regularly from 7 February onward (≥22.0 C).

March 1994 Spawns

By 13 March 1994, two of the females had noticeably swollen abdomens. On 23, 24, 25, and 26 March, the groupers spawned (Table 1). Light intensity was in the same range as in 1995 but was not measured. Full moon occurred 27 March at 0609 h. During the four spawning days, the

TABLE 1. *Voluntary spawning during March 1994 by two male and four female wild Nassau groupers held in a 37-m³ concrete raceway for 15 mo (salinity was 27.5–29.5 ppt).*

Spawn number	Date	Spawn time [period][a]	Hours before full moon	Water temperature (C)	Number of males spawning	Number of females spawning	Number of eggs (1,000s)	Fert. rate (%)	Hatch rate (%)
94-1	23 Mar	[1730–1930]	84	26.2	2	3	3,770	84	96
94-2	24 Mar	[1730–2100]	60	26.3	2	4	3,110	97	92
94-3	25 Mar	[1700–2100]	36	26.4	2	4	3,110	100	90
94-4	26 Mar	[1715–2040]	12	27.1	2	2–3	296	97	90

[a] U.S. Eastern Standard Time; sunset was at 1834 h.

broodfish showed no interest in eating seafood or pellets. They did not respond at all to as many as seven people walking along the top of the tank wall. Normally, when one or more people were on the wall, at least some of the fish swam to them. This behavior resumed the day after spawning ceased. During the first three spawning days (23–25 March), the two males became bicolored by about 1300 h and remained so until spawning was finished that night. Females became bicolored near the time of ovulation (between 1700 and 1730 h) as a signal of readiness to the males. Only three of the four females participated on day 1, but all four did during days 2 and 3. On 26 March (day 4), the males were bicolored for brief periods during late morning (e.g., 1020 h) and afternoon, but did not stay bicolored continuously until after 1700 h for one and 1717 h for the other. Two females became bicolored at 1717 h and definitely spawned, while a third was bicolored at times and appeared to spawn. On day 4, activity was much less intense and egg production was low. The next day (27 March), the males were not bicolored during the afternoon. Three females and the less-active male ate fish and pellets. The larger, more-active, male became bicolored at about 1700 h and began approaching females. The females reacted defensively, often with fin-spreading displays. After each rejection, the male first reverted to normal color, then after noticing another female, became bicolored and approached her only to be rejected again. The smaller, less-active, male became partly bicolored for a few seconds at a time but was not seen approaching females. By 1900 h, this activity had ceased and all fish were resting. No eggs were found in the tank. On 28 March, no courtship activity was seen and all fish were eating. By 31 March, all six fish were eating very well.

On spawning days, courtship activity included close frontal male to female approaches, nudging and butting by the males, and a kind of herding of females one at a time by one or both males. Before females became bicolored, they did not respond much to the males, but after bicoloration, they became very active. Once females were bicolored, all bicolored fish began swimming vigorously from end to end of the raceway, with brief stops during which males nudged and pushed females sideways, usually head to head. Parallel swimming of one female with one or both males was frequent, and all the other bicolored fish usually followed them.

Spawning began soon after females were bicolored. One female and one or both males participated each time. When gamete release occurred, one or both males were pressed tightly against the female. If only one male was involved, the pair turned sharply in a circle in the female's direction. If both males were involved, the three fish swam nearly straight but the males pressed so tightly that the female often was lifted partly out of the water. While one female

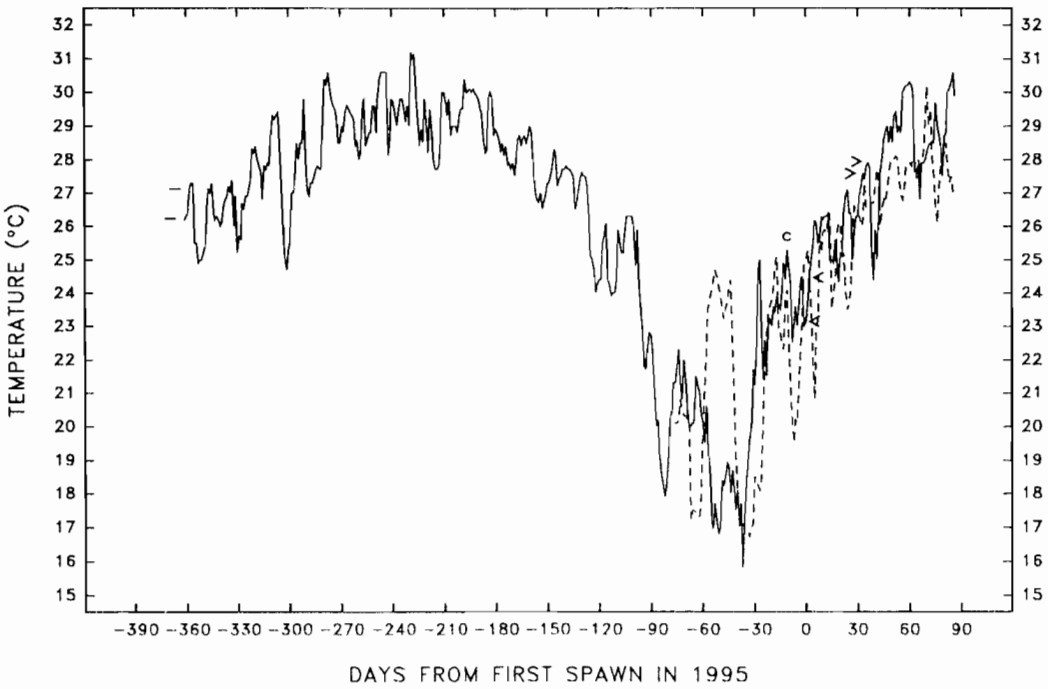

FIGURE 2. *Water temperature for captive wild Nassau grouper broodfish in a concrete raceway from 23 March 1994 to 12 June 1995 (solid line). The "-" (hyphen) symbols indicate first and last spawning days in 1994 (23–26 March). In 1995, courtship was first seen at the temperature peak below the "c" (4 March). The "<" and ">" symbols indicate first and last spawning days in 1995 (18–20 March, 2 ♀, 2 ♂; 20–23 April, 3 ♀, 2 ♂). The dashed line indicates water temperature during 1 January to 12 June 1996*

was spawning, the other bicolored females usually followed the spawning group closely. While bicolored, and especially when near a female, the males kept their dorsal fins extended. Bicolored females kept their dorsal fins retracted (apparently a submissive gesture), but after a female reverted to normal colors, when a male approached, she extended her dorsal spines and sometimes rammed or bit the side of the male. Females appeared to release eggs more than once per daily session, but this could not be confirmed in 1994. Activity continued until after dark, but slowly diminished until all fish had regained normal coloration and had taken their resting places for the night (between 1930 h and 2100 h).

Observations between Spawning Periods (1994–1995)

Courtship activity was not seen again until 22 d later on 17 April (full moon on 25 April). During 1800–2000 h, both males often became bicolored temporarily. As on the day after the last spawn in March, the large male chased and charged females. The females responded with defensive postures, usually with fins extended. If the male approached too closely, he sometimes was bitten on the side. The small male was much less aggressive and at most would swim slowly alongside a female. The females had slightly swollen abdomens, but never turned bicolored. By 2000 h, all the fish were resting. On 18 April, this behavior was repeated during 1830–2000 h. No eggs were found. Salinity was 34 ppt during 17–18 April. Water temperature was 26.9–27.2 C and did not exceed 27 C again until May. Subsequently, temperature remained higher than 27 C through the summer, and no further courtship behavior was observed until March 1995.

TABLE 2. *Voluntary spawning during March 1995 by two male and two female wild Nassau groupers held in a 37-m³ concrete raceway for 27 mo (salinity was 31.0 ppt).*

Spawn number	Date	Spawn time first[period][a]	Hours after full moon	Illuminance[b] (lux)	Water temperature (C)	Number of males spawning
95-1	18 Mar	~1800[1730–1830]	45.5	2,443	23.1	2
95-2	19 Mar	1811[1715–1811]	69.8	915	23.7	2
95-3	20 Mar	1700[1640–1900]	92.5	5,597	24.4	2
95-3	20 Mar	1729[1640–1900]	93.0	5,059	24.4	2

[a] U.S. Eastern Standard Time; sunset was at 1832 h.
[b] At the water surface under the greenhouse roof.
[c] Egg data for both females together.

Temperature dropped more slowly in fall 1994, going from 26 C to 23 C during 13 November to 13 December (30 d), but the fluctuations were wide, and it then took only 8 d to drop from 23 C to 20 C (Fig. 2). Next, temperature stayed below 23 C for 58 d. The annual minimum of 15.8 C was reached on 9 February 1995, 37 d before the first spawn. During 18 February–17 March (27 d), temperature was in the range 21.4–25.3 C. Food consumption was negligible during 30 January–12 February (15.8–19.0 C), started on 13 February (19.6 C), and increased greatly beginning 19 February (≥21.4 C). No courtship behavior was seen during December through February. On 4 March (water temperature 23.9 C), a bicolored male was seen herding one of the females in a circle. Duration of bicoloration and activity increased until 10 March, when the temperature dropped from 24.4 to 22.5 C after passage of a cold front.

March 1995 Spawns

Activity remained low until 18 March 1995, when at 1830 h both males and the larger of the two females were found to be bicolored. The stage of eggs sampled indicated that a small spawn had occurred at 1800 h, plus or minus 10 min (Table 2). By about 1900 h, the fish were normally colored and had settled down. Full moon had occurred 16 March at 2026 h.

On 19 March, both males remained bicolored from 1300 h until after 1900 h. The large female (72 cm) became bicolored at 1715 h (water surface illuminance = 5,300 lux) and began swimming with the two males, with vigorous rushes occurring at 1727 h and 1729 h and slightly weaker ones later. The three fish spawned at 1811 h (915 lux), whereupon the female immediately changed to normal colors and retired to her corner. The large male approached her several times and was repelled. Both males continued to patrol the length of the raceway, occasionally chasing the small female, and all four began settling down for the night at 1900 h (1 lux).

On 20 March, both males were bicolored from 1315 h until after 1900 h. Both females became bicolored just before 1700 h. The 72-cm female spawned with both males at 1700 h, and the 62-cm female did so at 1729 h. All four fish remained bicolored and continued to swim from end to end of the raceway in twos, threes, or all together until about 1900 h when they settled down.

On 21 March, the males remained bicolored from 1530 h until 1915 h. The large female was bicolored for only 5 s at 1651 h and repelled every advance by the males. The small female was bicolored for only 10 s at 1845 h and occasionally swam with one or both males. At 2045 h the males were

TABLE 2. *Extended.*

Total length of females spawning (cm)	Number of eggs (1,000s)	Egg diam. (μm)	Oil globule diam. (μm)	Fert. rate (%)	Hatch rate (%)
72	136	998	260	94	91
72	118	978	233	98	100
72	918	964[c]	238[c]	100[c]	95[c]
62	488	964[c]	238[c]	100[c]	95[c]

normally colored but were still swimming around. No spawning was witnessed, and no eggs were found in the tank.

All four fish resumed eating on 22 March and were eating very well by 26 March. Temperature reached 26 C on 23 March. The next full moon occurred 15 April at 0708 h and the last quarter 21 April at 2218 h.

April 1995 Spawns

Spawning in April 1995 began 30 d after the last March spawn (Table 3). On 20 April, the 62-cm female spawned a small number of eggs (probably less than 100,000), but interruptions prevented full documentation.

On 21 April, the 62-cm female turned bicolored at about 1800 h, spawned once at 1839 h with both males, continued to swim with them for 8 min, then reverted to normal coloration and returned to her corner.

On 22 April, the 62-cm female turned bicolored and spawned within 60 s at 1824 h, then spawned at least eight more times in the next 2 h. The 72-cm female spawned at 1850 h and 1858 h. The previously isolated (66 cm) female spawned at 2009 h. Three other spawns occurred at 1830 h, 1839 h, and 2033 h, but the females were not identified.

On 23 April, the 72-cm female spawned at 1832 h and the 66-cm female at 1840 h. Other spawns occurred at 1906 h and 1958 h. Spawning was not nearly as vigorous as the day before.

On 24 April, water temperature was 27.7 C, and no courtship, spawning, or eggs were seen. Temperature was in the range 24.4–28.5 C until 30 May. After 29 April, temperature remained higher than 27 C (except dropping once to 26.8 C on 23 May) and no further courtship behavior was observed during 1995.

Behavioral patterns were similar to those described for 1994. One exception was that the bicolored fish did not swim together as continuously as the year before. Also, the female not spawning on 23 April 1995 (62 cm) rammed and bit the previously isolated female (66 cm) during that session, interfering with fertilization. In 1995, we found that bicolored fish were so oblivious to outside distractions that when we gently poked or laid our hands on their backs during courtship and spawning, they did not hesitate or look up. When measured and weighed on 1 June, the males were still swollen and running ripe, but the females were not swollen.

1996

The groupers were watched every afternoon but did not spawn during spring 1996. The only observed activity related to spawning was bicoloration of the small male for a few seconds at 1840 h on 23 March. Full moons occurred on 5 March and 3 April. A likely factor was more extreme temperature fluctuations during February to April of that year compared to the previous two years (Figs. 1, 2). For the 15 d before spawning, water temperature was in the range 22.9–25.8 C for March 1994 and 22.5–25.3 C for March 1995. During the 30 d between the March and April 1995 spawns, water temperature was in the range 24.3–27.2 C. On 13 February 1996, the annual minimum was 16.7 C, a degree higher than in the previous two years. During each 15-d period from the second half of February through April 1996, water temperature was in the ranges: 18.1–25.0 C, 19.5–25.1 C, 20.8–25.9 C, 23.5–26.6 C, and 26.0–27.7 C. Steady temperature increase

TABLE 3. *Voluntary spawning during April 1995 by two male and three female wild Nassau groupers held in a 37-m³ concrete raceway for 28 mo (salinity was 29.0–29.5 ppt).*

Spawn number	Date	Spawn time first[period][a]	Hours after full moon	Illuminance[b] (lux)	Water temperature (C)	Number of males spawning
95-4	20 Apr	~1830[1800–1900]	131.4		27.6	2
95-5	21 Apr	1839[1800–1847]	155.5	217	27.4	2
95-6	22 Apr	1824[1824–2050]	179.3	703	27.8	2
95-7	23 Apr	1832[1815–2058]	203.4	266	27.9	2

[a] U.S. Eastern Standard Time; sunset was at 1849 h.

[b] At the water surface under the greenhouse roof.

[c] Because of an air conditioner breakdown, incubation was not possible. Hatch rates were similar to those of previous months.

[d] Egg data were not obtained.

[e] The 62-cm female did not spawn but rammed and bit the 66-cm female repeatedly during the spawning period. This could have reduced fertilization by causing some eggs to be released at the wrong time. The unfertilized eggs appeared normal.

or decrease, followed by a long enough period of temperature stability, could be important for voluntary spawning.

General Discussion

Typically in nature, Nassau groupers aggregate near a prominent section of the reef and later move slightly deeper to spawn in a depth range of 21–40 m (Colin 1992; Tucker et al. 1993). Spawning usually occurs during a rapid upward swim, sometimes halfway to the surface, followed by an equally rapid descent. Although in water only 0.9 m deep, the groupers in the raceway followed an urge to swim fast but could do so only horizontally. Then, rather than spawning in midwater, they spawned nearly horizontally with their backs out of the water.

In this study, bicoloration timing for both males and females was similar to that of fish spawning in natural aggregations (Colin 1992). In earlier laboratory work beginning in January 1987, both injected (human chorionic gonadotropin) and uninjected newly-caught females in 1,000-L tanks often became bicolored near the time of ovulation (Tucker and Woodward, in press). However, the newly-captured males became bicolored at about the same time as the females, rather than 4 h before as for long-term captive males in the raceway. When eggs were released in the short-term holding tanks before females were removed for stripping, fertilization was always near 100%.

In nature, Nassau groupers school as juveniles but are solitary as adults, except when aggregating for spawning. Even only four wild adult fish in a 37-m³ raceway occasionally had aggressive encounters. Two males might be better than one because their interaction and frequent bicoloration seem to encourage the females, but a third male could be superfluous and might get in the way or be excluded during spawning in a shallow tank. The number of females might not matter as much. Because males ripen easily and the female that had been isolated for 6 mo (66 cm) spawned 4 d after rejoining the other fish, prolonged social interaction seems unnecessary for ripening of males or females.

During the spawning season, captive males had a slightly but obviously distended abdomen. Females sometimes did and sometimes did not, probably depending on the number of mature oocytes present and the degree of hydration. Males can have a gonadosomatic index as high as 16.4% and

TABLE 3. *Extended.*

Total length of females spawning (cm)	Number of eggs (1,000s)	Egg diam. (μm)	Oil globule diam. (μm)	Fert. rate (%)	Hatch rate (%)[a]
62	Small[d]				
62	1,036	972	243	97	High
62, 66, 72	5,217	945	231	100	High
66, 72	3,589	984	230	83[e]	High

females 18.7% (Tucker et al. 1993). Each million released eggs occupies a volume of about 900 mL.

Salinity at spawning sites in the Cayman Islands and Bahamas typically is in the range 35–37 ppt (J.W. Tucker, personal observation; Pitts and Smith 1993). Eggs obtained from induced and natural ovulation require a salinity of 30–32 ppt to float. Lower than normal salinity of 27.5–31.0 ppt in the raceway did not seem to limit spawning. The eggs were suspended rather than floating when salinity was low, but they floated when transferred to 31 or 32 ppt water in beakers and small tanks.

Daylength during laboratory spawning was 12.0–12.9 h. For field spawning, daylength ranged from 10.5 to 14.3 h and, in different areas, was the shortest, increasing, or the longest (Tucker et al. 1993).

In nature, Nassau groupers seem to spawn mostly from a few days before the full moon until near the last quarter moon (Colin 1992; Tucker et al. 1993), but spawning also can occur near the new moon. During late afternoon of 10 December 1993 (2.5 d before new moon), we observed several females that had been caught 2–4 d earlier ovulate viable eggs in holding cages in Nassau. Of more than 100 females examined the day before and that morning, all were close to full ripeness and none had ovulated recently. In this study, spawning occurred from 3.5 d before until 8.5 d after the full moon (near the last quarter). The full moon in March 1995 was 10.4 d earlier than in 1994, and the first spawn was 5 d earlier than in 1994. Both the shifted moon phase and less temperature fluctuation in 1995 could have been factors leading to the two spawning periods.

Sunset was at 1832–1849 h and egg release occurred between 1700 h and 2100 h with initial illuminance of 217–5,597 lux and some spawns occurring at <0.1 lux. In earlier work with newly caught females held in 1,000-L tanks, natural ovulation (N = 12) always occurred during 1600–2000 h local standard time, under fluorescent lighting with some horizontal natural light coming from under the roof (Tucker and Woodward, in press). Colin (1992) reported observations of natural spawning at 21–35 m depth during 20 min before until 20 min after sunset. Tucker et al. (1993) reported voluntary spawning in a cage at 9 m about 1 h after sunset. Other species also spawn late in the day. For example, the Malabar grouper *E. malabaricus* spawned in uncovered concrete ponds during 1600–2300 h (L.-T. Lin, Tung Shing Aquatic Hatchery, Ping Tung Hsin, Taiwan, ROC, personal communication, 1992).

Each female Nassau grouper spawned for 1–4 d. Females of other species are known to reproduce over a period of days. For example, J. D. Toledo (Fisheries Department, Bandar Seri Begawan, Brunei Darussalam, personal communication, 1992) reported that a single female *E. coioides* with two males spawned 5–10 times a month for 4 mo.

Nassau groupers usually begin natural spawning: 1) in the Bahamas, Mexico, and Belize in November or December when temperature is slowly decreasing; 2) at St. Thomas, Cayman Islands, and Jamaica in January or February when temperature is lowest; and 3) near Bermuda in May when temperature is slowly increasing (Tucker et al. 1993). Spawning at the first six locations continues through the colder months, and in Bermuda it continues through the warmer months. The usual temperature range during the spawning season is 23–27 C, and

temperature during spawning usually is in the range 25–27 C (Colin 1992; Tucker et al. 1993).

The experimental broodstock were from a population that normally spawns in December and January. Spawning of Nassau groupers can occur in different parts of the Bahamas from late November through at least March (Colin 1992; Tucker, personal observation). In many spawning areas, water temperature does not often drop below 23 C in the winter, and the expected range is 24–26 C (Colin 1992; Pitts and Smith 1993). In Nassau, during December 1992, January 1993, February 1993, early and late December 1993, January 1994, and January 1995, we induced ovulation in 19 females with human chorionic gonadotropin (Tucker and Woodward, in press). Males were not injected. Water temperature range in the laboratory was 23–25 C. During those same months, 12 newly-captured females ovulated naturally in tanks without treatment.

Temperature conditions in Florida are intermediate. Spawning temperatures occur in both the fall and spring, but might not persist long enough in the fall for full oocyte development. Like in the raceway, natural waters off Florida experience a rapid temperature decrease in the fall and a more gradual increase in late winter. Species such as red grouper *Epinephelus morio*, gag *Mycteroperca microlepis*, and black grouper *M. bonaci* spawn in Florida waters mainly during February to April when temperature is gradually increasing.

Nassau groupers are very flexible with regard to spawning season and seem to be stimulated mainly by temperature. Although the natural spawning season at a given location usually lasts only 2–3 mo, this species has been reported to spawn every month except September and October (Tucker et al. 1993; J. W. Tucker, personal observations and interviews with fishermen). The captive wild groupers easily adapted to local conditions and spawned 3 mo later than they would have in their home waters. The most likely cause for delay of spawning was the rapid temperature decrease during November and December. When water temperature drops through the spawning range too rapidly, time is too short for complete oocyte development. If the temperature stays in a suitable range, females will hold their oocytes at an intermediate stage until the narrower spawning temperature range is reached and persists long enough for final maturation and ovulation.

It is likely that initial and final spawns in 1995 were near the temperature limits for success. Fertilized Nassau grouper eggs experience more developmental anomalies when incubated outside the range 23–27 C (personal observations over several seasons). Two days before spawn 95-1 (Table 2), water temperature rose to 24.5 C, but the next day, it dropped to 23.0 C. Spawns 95-1 and 95-2 were much smaller than usual. It is possible that the 72-cm female was too close to ovulating to hold back the eggs completely, but did hold most of them (78%) until spawn 95-3 when temperature had risen to 24.4 C. The 62-cm female spawned on the third day only. During the 2 d before spawn 95-4 in April (Table 3), temperature was 26.9 C and 27.2 C, the highest yet for the year. It is possible that the fish were conditioned to ovulate at temperatures of $\leq$27 C and followed through despite the slightly high range of 27.4–27.9 C on spawning days.

Nassau groupers, like many other serranids (Tucker 1994) will spawn in captivity if protected from stress (especially crowding and handling), fed well, and taken through a suitable temperature cycle, even if secondary factors are not optimal (e.g., low salinity). The month of spawning depends on temperature and can be shifted. Gradual increase of temperature to 23–24 C or decrease to 26–27 C seems to be the most important environmental factor. We believe Nassau groupers can be conditioned to spawn voluntarily any month of the year.

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