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research paper

Diel, lunar, and seasonal spawning patterns of the Atlantic goliath grouper, *Epinephelus itajara*, off Florida, United States

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ABSTRACT.—The diel, lunar, and seasonal timing of spawning in Atlantic goliath grouper *Epinephelus itajara* (Lichtenstein, 1822) in the United States is highly specific, occurring at night during new moon phases of August, September, and October. We derive these patterns from four lines of evidence apparent on spawning sites during the known spawning season: (1) from the transitory appearance of fish aggregations; (2) from simultaneous recordings of goliath grouper nighttime calls and nighttime vertical ascents that were far more frequent during the new moon phase than on the full moon; (3) from collections of goliath grouper eggs (genetically verified) at night downstream from known spawning sites; and (4) from significantly higher frequencies of both hydrated oocytes (indicating imminent spawning) and postovulatory follicles (indicating recent spawning) in ovarian biopsies taken from goliath grouper captured on spawning sites during new moon phases relative to full moon phases. We suggest that dark-night spawning is an adaptation minimizing egg predation by several species of scad [*Decapterus punctatus* (Cuvier, 1829), *Decapterus tabl* Berry, 1968, and *Decapterus macarellus* (Cuvier, 1833)] and herring [*Sardinella aurita* Valenciennes, 1847 and *Etrumeus teres* (DeKay, 1842)] that are abundant on goliath grouper spawning sites. The seasonal spawning of goliath grouper, late summer–early fall, coincides with habitat conditions considered ideal for settlement of early juveniles in mangrove nurseries.

Many reef fishes, including groupers (Epinephelidae), exhibit reproductive patterns that parallel lunar cycles with spawning occurring near dusk (Johannes 1978, Thresher 1984, Colin et al. 1987, Shapiro 1987, Sadovy 1996, Domeier and Colin 1997, Rhodes and Sadovy 2002, Sadovy de Mitcheson and Colin 2012). Many large groupers tend to make extensive seasonal migrations to form spawning aggregations on traditional sites to attend reproductive events that persist for days to months (Colin et al. 1987, Shapiro 1987, Coleman et al. 1996, Zeller 1998, Rhodes and Sadovy 2002, Ellis et al. 2014). This behavior is common among larger species, but not smaller ones (Sadovy 1996). Aggregation size among grouper species varies, with some forming many small (<100 individuals) aggregations distributed over a wide area (Coleman et al. 1996), including gag [*Mycteroperca microlepis* (Goode and Bean, 1879)] and scamp (*Mycteroperca phenax* Jordan and Swain, 1884), whereas others, such as Nassau grouper [*Epinephelus striatus* (Bloch, 1792)], form very large aggregations (thousands of individuals) at only a few sites (Smith 1972).

Spawning aggregation characteristics of Atlantic goliath grouper *Epinephelus itajara* (Lichtenstein, 1822) (hereafter "goliath grouper")—the largest grouper in the Atlantic Ocean—has been difficult to determine until recently. The first directed observations of their aggregations off the southeastern US, made by Colin (1994), occurred when the population was at an all-time low (due to intensive overfishing) and aggregation size was quite small (<10 individuals), making it difficult to discern either diurnal or lunar reproductive patterns. It was not until the population started to recover, which we recently documented (Koenig et al. 2011), that a realistic evaluation could be conducted. In addition to documenting the initiation of population recovery, we have elucidated aspects of spawning migrations, spawning locations, and timing using acoustic telemetry and passive acoustics (Koenig and Coleman 2013). We now know that goliath grouper form multiple small (<100 individuals) spawning aggregations drawn from areas sometimes hundreds of kilometers away from spawning sites (Koenig et al. 2011, Ellis et al. 2014).

In this paper, we describe the seasonal, lunar, and diel timing of spawning of goliath grouper in Florida and discuss these temporal patterns as driven by various aspects of adult, larval, and juvenile biology (review by Robertson 1991).

Materials and Methods

Spawning Sites.—We relied heavily on the local knowledge of commercial fishers and divers to locate spawning sites of goliath grouper. Some of these fishers had previous experience targeting this species prior to the fishery closure in 1990. We confirmed spawning aggregation sites through: (1) direct observation of increases in population size via scuba diving; (2) passive acoustic monitoring; (3) histological analysis of ovarian biopsies; and (4) nighttime net sampling of goliath grouper eggs. We also used acoustic telemetry with the involvement of the Florida Atlantic Coast Telemetry (FACT) group initiated and coordinated by the Florida Fish and Wildlife Conservation Commission (see Ellis et al. 2014 for details), and through the use of the Reef Environmental Education Foundation (REEF) volunteer dive surveys (http://www.reef.org/programs/volunteersurvey) (Koenig and Coleman 2009, 2013).

Passive Acoustic Monitoring.—We recorded goliath grouper sounds (low frequency sounds that occur within the range of 0–100 Hz) using DSG-Ocean acoustic recorders (http://loggerheadinstruments.com/) placed on known or suspected goliath grouper spawning aggregations off southwest and southeast Florida during the 2010, 2011, and 2012 spawning seasons. The DSG-Ocean, a low-power underwater acoustic recorder that makes high-quality acoustic recordings over long periods, records to SD memory cards using a FAT32 file system. The DSG-Ocean can be programmed to either sample continuously, at rates approaching 80 kHz, or intermittently to conserve battery power.

We made all acoustic recordings on well-known shipwrecks, most of which were intentionally deployed as artificial reefs by state and county agencies. On the Atlantic coast (off Palm Beach County, Florida), recordings were made on the MG111, Zion Train, and Gulfland wrecks, all located within 10 km of each other. Water depths for these sites are 9 m (Gulfland), 20 m (MG111), and 28 m (Zion Train). In the Gulf of Mexico, we made recordings off Lee County on the Fantastico (35 m deep, a freighter that sank in a storm in 1993) and the Stoney wreck (40 m deep), both known spawning sites (Koenig and Coleman 2013).

We attached acoustic recorders directly to the wrecks during the spawning season (late August or early September), where they remained for several months (through late November), recording intermittently for 10 s every 10 min within a frequency range of 0–10 kHz. Analysis using a Fast Fourier Transform allowed us to examine the concentration of acoustic energy in the 0–100 Hz range and analyze using MATLAB R2009b and Adobe Audition 2.0.

Capture and Sampling of Live Fish.—Collecting samples to determine reproductive condition, age structure, diet, and degree of contamination of various toxicants required that we capture live goliath grouper. We tagged captured fish externally with cattle tags and internally with PIT (passive integrated transponders) tags. Some individuals were additionally tagged with acoustic tags (Vemco VR16 tags; 69 kHz, with 8-year batteries) surgically implanted intraperitoneally for determination of movements related to spawning activity (see Ellis et al. 2014). We followed federal and state laws for handling a protected species[1] that require the release of captured animals alive and in good condition. We captured goliath grouper during the spawning season on known or suspected spawning aggregation sites using hand lines (9-mm braided nylon, 60-m long) with monofilament leaders (1000-lb test, 5-m long) and circle hooks (20/0). Bait included whole live hardhead catfish [*Ariopsis felis* (Linnaeus, 1766)], and 1–2 kg pieces of great barracuda [*Sphyraena barracuda* (Walbaum 1792)], greater amberjack [*Seriola dumerili* (A. Risso, 1810)], little tunny [*Euthynnus alletteratus* (Rafinesque, 1810)], or stingrays [*Dasyatis sabina* (Lesueur, 1824) and *Dasyatis americana* Hildebrand and Schroeder, 1928]. The leader was connected to a hand line and to a 2-kg lead weight with longline snap (8/0 model 148, 1000-lb test). At times, we used a polyball buoy (69-cm diameter) to suspend the bait off the bottom and transfer the force of the powerful pull of the grouper to the buoy rather than the fisher's hands.

Each captured fish was hauled through a door in the vessel's transom onto a stretcher and tied down with straps to keep the fish from thrashing about the deck, protecting both fish and field personnel. We covered the exposed eye with a damp towel protecting it from direct sunlight and from responding to visual stimuli. A

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[1] Goliath grouper are protected from all forms of fishery extraction in all United States continental and Caribbean territories.

hose with constantly flowing seawater inserted into the mouth irrigated the gills continuously.

We vented each fish on deck just posterior to the pectoral fin and below the midline with a stainless steel trocar (9.5-mm diameter) and cannula (http://www.scbt.com/datasheet-362154.html). The trocar and cannula were inserted through the body wall into the swimbladder, then the trocar was removed leaving the cannula inserted for about 1 min or until the gas had fully escaped the swimbladder. All fishing occurred at depths less than approximately 35 m to limit the effects of barotrauma, which can cause hemorrhage and death at greater depths of capture.

Gonad Biopsies.—We took gonad biopsies of all captured fish during the spawning seasons of 2010, 2011, and 2012 to determine sex and reproductive condition. The gonad biopsy method used on captured females involved inserting a polyethylene catheter (6.3-mm OD, 4-mm ID) through the oviduct into the lumen of the ovary and removing tissue with a hand-operated vacuum pump (Mityvac MV8000). Drawing the inserted tube back and forth in the lumen of the ovary allowed continuous removal of ovarian tissue. We withdrew the tube while still under vacuum to draw the ovarian tissues into a collection cup inserted in the vacuum line. We preserved half of the gonad tissue in 10% formalin for histological studies and half on ice for subsequent freezing followed by analysis of toxicants, such as mercury. Formalin-preserved samples were allowed to fix for several days, after which the individual tissue samples were placed in plastic tissue cassettes, washed with 70% ethanol, put into plastic bags, sealed, and shipped to Crowder Histology Consulting (4952 Alvin Dark Ave., Baton Rouge, Louisiana 70820) for preparation of histological slides.

We modified the biopsy method for males by using a smaller diameter catheter (2-mm OD) and/or human uterine biopsy forceps. The small diameter of the sperm duct made biopsies particularly difficult to obtain from males.

Only one reader (DJM) analyzed all of the histological sections. For our study, the most important features identified were hydrated oocytes and recent (i.e., newly formed) post-ovulatory follicles (POFs). The hydrated egg and POF data were standardized to percent occurrence and displayed relative to moon phase at the time of capture (representing time of the biopsy). We analyzed the frequency of occurrence across moon phases using a chi-square test and tested for significant differences of POFs among lunar phases using the Marascuilo comparison procedure (Marascuilo 1966).

Nighttime Egg Collection.—We set plankton nets (0.5-mm mesh) downstream from active spawning sites (location of fish determined by echosounder or diver observation) off Palm Beach County, Florida, to capture eggs of goliath grouper. To locate downstream deployment sites, we released a drogue (a weighted 1-m diameter sea anchor attached to a float) where the aggregated fish were observed, and then followed it 50–100 m downstream, anchoring mooring lines with nets attached at the end point and noting coordinates.

Three mooring lines were separated at 10–15 m intervals; buoyed at the surface, anchored to the bottom, and aligned perpendicular to the current, these served as attachment sites for several nets on each line distributed from the surface to about mid-depth in the water column. Mooring line anchors were rigged to break away from the bottom with sufficient tension from the vessel; this facilitated retrieval of the nets in the strong Florida Current.

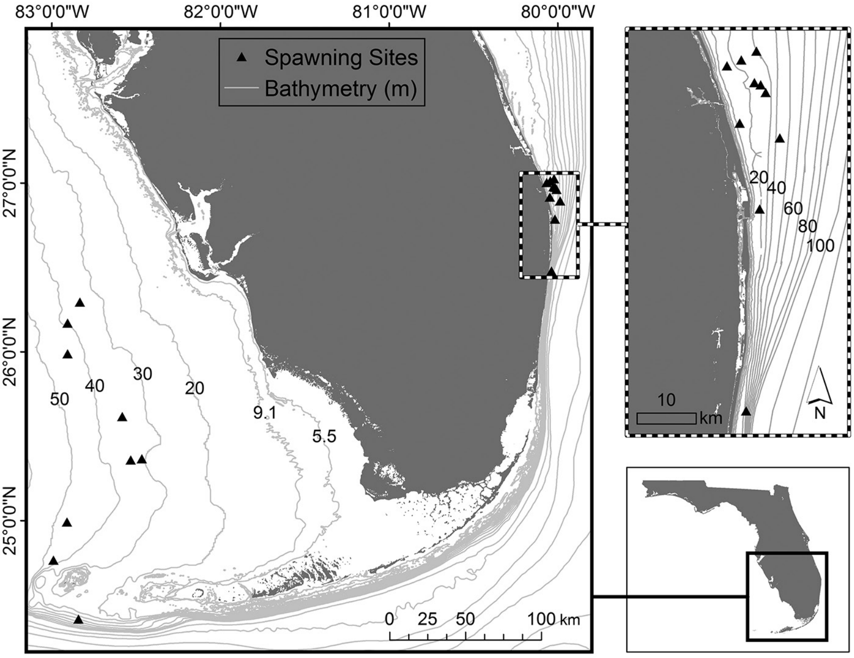

Figure 1. Map of confirmed Atlantic goliath grouper, *Epinephelus itajara*, spawning sites in Florida. Left panel: sites off southwest Florida and southeast Florida coasts. Right upper panel: off Palm Beach County. Right lower panel: entire state of Florida.

We deployed nets soon after sundown and left them out all night during the first deployment. We stopped using this approach when we determined that nets fouled quickly with plankton and debris, decreasing their capture efficiency. Subsequent deployments occurred at sundown with retrieval just before midnight, approximately 4–6 hrs after deployment.

Once retrieved, nets were held over a 5-gallon bucket and washed gently on the inside to avoid dislodging plankton and small jellyfish embedded in the mesh (intense washing of the nets made separation of eggs from plankton very difficult). The samples were then poured through three nested sieves of different mesh sizes: 3-mm mesh and 1.5-mm mesh to remove unwanted material; and a 0.5-mm mesh to collect eggs (approximately 0.95 mm diameter). Washing eggs off the 0.5-mm sieve into a 5-gallon bucket filled with clean, high-salinity (35) seawater resulted in live eggs floating to the surface while most dead plankton and eggs sank. After about 20 min, we swept a small fine-mesh (0.5 mm) aquarium net around the inside edge of the buckets at the water surface and transferred the net contents to a second bucket filled with clean high-salinity seawater, repeating the process at least two times to remove dead plankton and debris from the sample of developing eggs.

Results

Spawning Aggregations.—We confirmed 20 locations as actual spawning sites based on fish densities, sound production, ovarian biopsies, observations of commercial fishers working prior to the fishing moratorium, and/or egg collection. This evidence was collected during and outside of the spawning season during this and

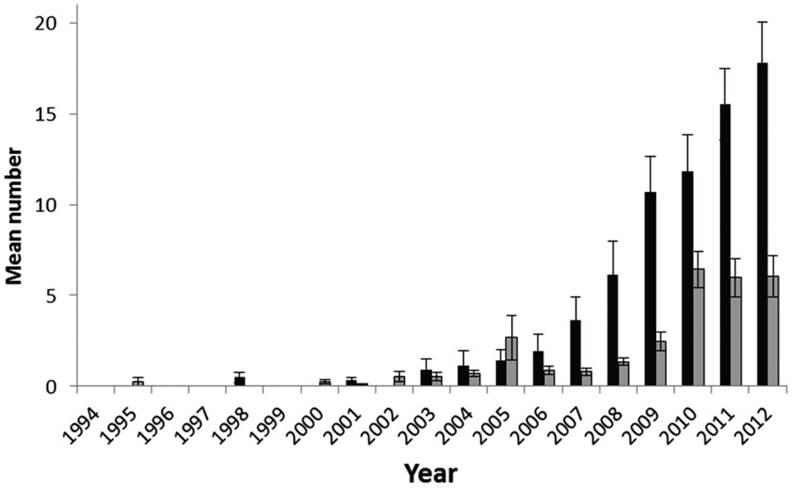

Figure 2. Annual mean abundance (±SE) of Atlantic goliath grouper, *Epinephelus itajara,* based on repeated surveys of all sites off the southeastern coast of Florida, from the southern Martin County through Palm Beach County, Florida, during (black bars) and outside (grey bars) of the spawning season (August through September). Because spawning and non-spawning sites were included in these REEF surveys the spawning season abundance (black bars) is underestimated.

our previous studies. Verified spawning sites occurred off the southeastern (SE) and southwestern (SW) coasts of Florida (Fig. 1). Spawning habitats consisted of relatively high-relief rocky reefs and artificial reefs (including wrecks and towers) in water depths of 15–50 m (Koenig et al. 2011). We found no spawning sites on or near coral reefs along the Florida Keys reef tract, an area in which the abundance of goliath grouper is relatively low (Koenig et al. 2007, 2011). There were sufficient data available from the REEF database to show the buildup of the goliath grouper population in the spawning area off SE Florida (Fig. 2), but not enough to demonstrate this pattern off SW Florida. Because all sites reported to REEF were used in Figure 2, including both spawning and non-spawning sites, the graph underestimates population increases during the spawning season.

Sound Production.—We recorded distinct patterns of nighttime calls ("booms", as described in Mann et al. 2009) off SW and SE Florida sites during the spawning seasons of 2010 and 2011. These calls were observed on spawning sites—Fantastico and Stoney wrecks off SW Florida and the MG111 off southeast Florida—but not on the non-spawning site, Gulfland wreck off SE Florida, where fish were less abundant and generally smaller than on known spawning sites (Fig. 3A–D).

Ovarian Biopsies.—We sampled 253 live goliath grouper captured off Palm Beach County, Florida, during the spawning seasons of 2010–2012 and analyzed histological preparations of ovarian biopsies for frequency of occurrence of hydrated oocytes and POFs (Fig. 4A, B) on all females: 2010 (n = 30 females), 2011 (n = 37 females), and 2012 (n = 94 females) (total n = 161, including recaptures). Ovarian biopsies indicated significantly higher frequency of occurrence of POFs (χ^2 test: $P <$ 0.0001) and hydrated oocytes (χ^2: $P < 0.05$) in samples collected during the new moon phase than in those collected during the full moon phase (Fig. 5A, B). Because of the small sample size of females captured and examined during first quarter (n = 9) and

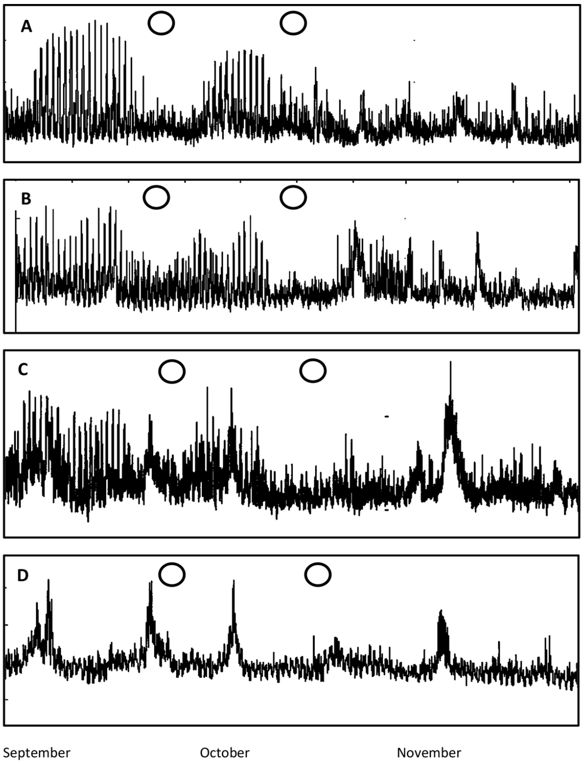

Figure 3. Patterns of nocturnal sound production in Atlantic goliath grouper, *Epinephelus itajara,*on three spawning sites: (A) Stoney Wreck, 2010, Gulf of Mexico; (B) Fantastico Wreck, 2010, Gulf of Mexico; (C) MG111 wreck, 2011, Atlantic Ocean; and one non-spawning site, (D) Gulfland wreck, 2011, Atlantic Ocean. Open circles = full moons of September and October. Lunar phases of August are not represented. Each new moon peak represents the nightly maxima of sound production levels (band sound pressure level dB re 1μPa (0–100 Hz); therefore periods associated with peak to peak intervals are approximately 24 hrs.

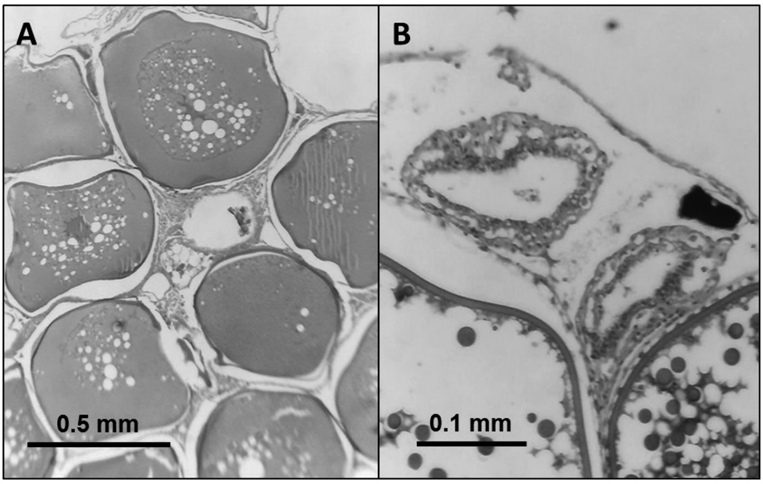

Figure 4. Photomicrographs of histological sections of ovarian tissue in Atlantic goliath grouper, *Epinephelus itajara,* captured off the southeastern coast of Florida, during the new moon of spawning season. Shown are (A) hydrated oocytes and (B) recent postovulatory follicles (POFs)—note double cell layers on POFs.

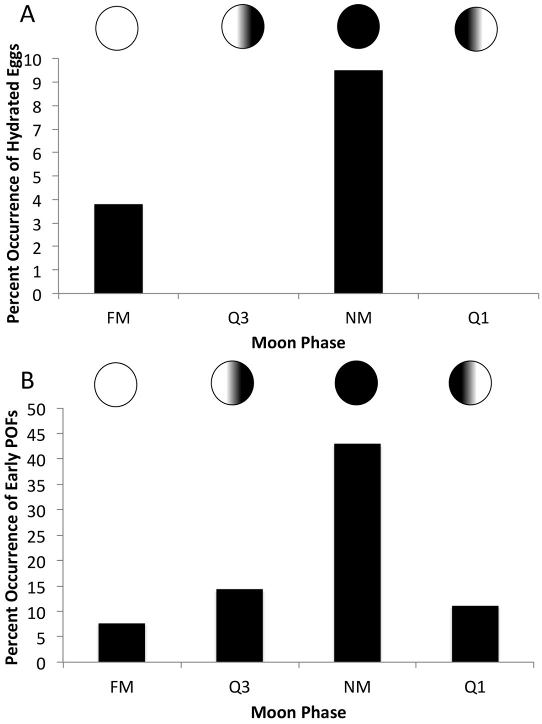

Figure 5. Comparison of changes in gonad characteristics relative to moon phase in female Atlantic goliath grouper, *Epinephelus itajara,* sampled in 2010, 2011, and 2012 on spawning aggregations off southeastern Florida (near Jupiter). Shown are (A) percent occurrence of hydrated oocytes and (B) percent occurrence of early postovulatory follicles (ePOFs). Description of moon phases are as follows: New Moon (NM; closed circle) = NM ± 3.5 d; and First Quarter (Q1) = NM ± 3.5 to NM ± 11.5 d. Full Moon (FM; open circle) = FM ± 3.5 d; Third Quarter (Q3) = FM ± 3.5 FM+11.5 d. New moon occurrences were significantly greater than full moon: χ^2 tests for POFs, $P < 0.0001$; for hydrated oocytes, $P < 0.05$.

third-quarter moon phases ($n = 7$), we could not distinguish them from new moon or full moon samples (Marascuilo comparison: $P > 0.05$).

Male Biopsies.—Male biopsies were largely unsuccessful. Identifying reproductively active males, however, was not. All mature males captured on spawning aggregations released copious quantities of milt. When the expansion of gas in the swimbladder caused fish to roll on their backs as they surfaced, milt spewed upward a meter or more in the air.

Egg Collection.—We collected several thousand goliath grouper[2] eggs during the new moon of September 2008 (Fig. 6) downstream from spawning sites off SE Florida (Palm Beach County, Hole-in-the-Wall—a natural reef) and off SW Florida (Lee County, Fantastico). Similarly, we collected many eggs downstream of a spawning site off SE Florida (Palm Beach County, MG111) during the new moon phase of August 2012, providing additional evidence that these sites served as active spawning

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[2] Species identification verified genetically by M Craig using the methods described in Craig et al. (2009).

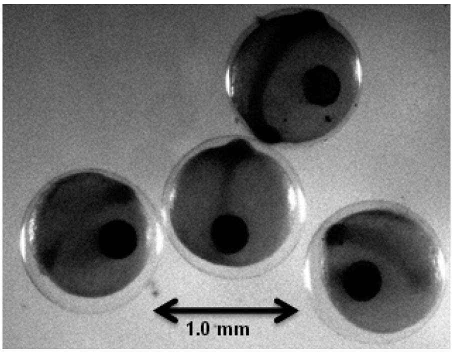

Figure 6. Photomicrograph of Atlantic goliath grouper, *Epinephelus itajara*, eggs in the neurula stage.

sites. All eggs were in early stages of development indicating that they were derived from nighttime spawns.

Direct Observation of Spawning.—J Hays (National Geographic underwater photographer) is the only person we know who has observed goliath grouper spawning. Notes on her observations, taken soon after sunset at a confirmed spawning site off Palm Beach County, Florida, in the new moon phase of September 2011, state that "The large presumptive female was tightly surrounded by large numbers of small planktivorous fish (scad and herring) [...] The presumptive female appeared to rub its vent on a vertical surface of the wreck, then swam away from the wreck and slammed its body against the sand then swam about erratically, in an apparent attempt to evade the many little fish, then she ascended straight up into the water column while followed by two smaller presumptive males. At the apex of their ascent, a cloud was seen (apparently a sperm cloud), then all three goliath groupers swam down to the sand and dispersed—the entire spawning event took less than 20 s."

Discussion

We know from previous studies that during their juvenile and adult life stages, goliath grouper show strong site attachment to their home ranges and to spawning sites (Koenig et al. 2007, 2011, Collins et al. 2015). For juveniles, the primary habitats are the mangrove forests that border south Florida rivers and islands, whereas for adults, they are rocky reefs, wrecks, or artificial reefs typically in shallower water for home sites and in deeper water for spawning sites. Home sites can also serve as spawning sites, as evidenced by fish numbers increasing dramatically during the spawning season. Our data and that collected by REEF bear this out (Koenig et al. 2011).

Behavioral and Morphological Characteristics of Active Spawners.—Goliath grouper reproduction in Florida strongly paralleled lunar patterns—migration to spawning sites occurred during the full moons of July, whereas spawning occurred with new moons of August, September, and October (Ellis et al. 2014, present study). During new moons, goliath grouper displayed distinct behavioral and, in the gonad, morphological characteristics. These included: (1) intense nocturnal calling (Mann et al. 2009, and present study); (2) the presence of hydrated eggs,

Figure 7. Photograph of Atlantic goliath grouper, *Epinephelus itajara*, adult on a spawning site off Palm Beach County, Florida surrounded by round scad (*Decapterus punctatus*) and Spanish sardines (*Sardinella aurita*). Photo credit: L Bueno, printed with permission.

which are indicators of imminent spawning (i.e., <12 hr); and (3) the presence of new POFs—indicators of immediate post-spawning[3].

We suspect for goliath grouper that in the absence of visual cues, calling serves as a means of aggregating potential spawners into a cohesive unit and providing critical behavioral cues that spawning is imminent, leading to synchronized hydration of eggs, gamete release, and maximized fertilization success (Lobel 2002). Sound production may also serve to stimulate hormone production associated with spawning condition (Locascio and Mann 2011). These findings support previous research on goliath grouper and other epinephelids demonstrating the relationship between fish sound production and seasonal patterns of reproduction (Mann et al. 2009, 2010, Nelson et al. 2011, Rowell et al. 2011, 2012, Schärer et al. 2012, 2014, Locascio and Burton 2016). They also suggest that nighttime calling is an effective means of identifying active spawning sites. Still unknown is whether the frequency of nighttime calls during new moon phases is a good indicator of the size of the spawning population. A recent study by Rowell et al. (2012) demonstrated this relationship holds true for red hind [*Epinephelus guttatus* (Linnaeus, 1758)] in Puerto Rico. If true for goliath

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[3] POF degeneration occurs quite rapidly in most fishes in warm water temperatures: within 10–12 h in the northern anchovy, *Engraulis mordax* Girard, 1854 (Hunter and Macewicz 1985), <24 hrs in Atlantic menhaden *Brevortia tyrannus* (Latrobe, 1802) (Fitzhugh and Hettler 1995); 24 hrs in red snapper *Lutjanus campechanus* (Poey, 1860) (Jackson et al. 2006). If goliath grouper have similar POF degeneration times, then full disappearance of POFs would occur well within a lunar phase.

grouper, then acoustic monitoring could be an extremely useful tool not only for determining on-site spawner density, but also for determining relative spawning stock biomass throughout its range in the southeastern United States.

While night spawning is clearly the dominant pattern in goliath grouper, we cannot exclude the possibility that daytime spawning also occurs. It seems extremely unlikely, however, because we have not observed a single spawning event during the thousands of hours we dived with goliath grouper in the daytime on spawning sites during the spawning season from 1994 through 2015. Nor have we received a single report from the many other divers we know, especially off Palm Beach County, Florida. If some daytime spawning does occur, it would likely be minor relative to nighttime spawning.

We also suspect that goliath grouper are group spawners, meaning multiple males ascend with a single female during a spawning event. We draw on two lines of evidence from this and our previous studies: (1) the observation that more than two fish participate in spawning rushes; and (2) the observation that males produce copious quantities of sperm. Sperm production of such a large magnitude is indicative of group spawning rather than pair spawning as sperm competition dominates over individual competition for fertilization (Petersen and Warner 1998). This is borne out in groupers by the difference in size of testes of pair spawners such as red grouper, *Epinephelus mori*o (Valenciennes, 1828), and gag, which have relatively small testes (Coleman and Koenig, pers obs) and group spawners, which tend to have large testes.

Egg Collection.—An additional approach to determine unequivocally whether the suspected goliath grouper aggregation sites were in fact spawning sites was to capture eggs during spawning events and genetically verify egg species identity. We assumed that this would be relatively straightforward because of our success in collecting fertilized goliath grouper eggs from the Hole-in-the-Wall natural reef on the first try in 2008 using a downstream passive sampling approach (Koenig and Coleman 2009). However, upon repeated attempts, we encountered numerous problems, including highly unpredictable current speeds and directions, and rapid fouling of nets caused by high densities of plankton, blooms of moon jellyfish (*Aurelia aurita* Linnaeus, 1758), and floating debris. Because this technique required ideal conditions, which rarely occurred, we determined that egg collection in this manner had limited applicability for verifying spawning sites.

Determinants of Seasonal Timing of Reproduction.—The timing of spawning in fishes (seasonal, lunar, and diel) is critical to reproductive success (Lowerre-Barbieri et al. 2011, Donahue et al. 2015). In broadcast spawners with pelagic larvae, a broadly accepted hypothesis is that reproductive success requires a match between the spawning season and optimal conditions for larval survival (Hjort 1914, Cushing 1943, and others). That is, larval biology controls reproductive timing. This idea has dominated analyses of seasonal patterns of spawning of temperate and tropical marine fishes (Robertson 1991). This belies the fact that natural selection also occurs on juveniles and spawning adults, not just larvae, in the scheduling of reproduction that ultimately maximizes reproductive success.

Successful recruitment to and survival in suitable settlement habitat is an important determinant of reproductive success (Lowerre-Barbieri et al. 2011). Juvenile habitat requirements include availability of refuge, abundant food, and suitable environmental conditions for optimal growth and survival. If the quality and quantity of settlement habitat varies seasonally, then the seasonal timing of spawning may be strongly influenced by the timing of optimum settlement conditions.

For goliath grouper off Florida, larvae derived from August through mid-October spawning events settle as juveniles from September to early January (pelagic larval duration is 30–80 d; Lara et al. 2009) in mangrove nurseries primarily in submerged mangrove leaf litter (Koenig et al. 2007, Lara et al. 2009). Litter fall in south Florida occurs year round, increasing in the autumn (Lugo and Snedaker 1974, Twilley et al. 1986, Dawes et al. 1999) and during the dry season (December through May) when salinity increases. Under conditions of increased salinity, leaf breakdown is faster and the community of macroinvertebrates more abundant (Odum et al. 1982). Settlement of goliath grouper just before or during the dry season may confer a survival advantage because: (1) they occur only in waters with higher salinities (Odum and Heald 1972, Koenig et al. 2007); and (2) the abundance of potential prey for the early stage juveniles is higher (Odum et al. 1982, Sadovy and Eklund 1999).

A similar relationship between the timing of spawning and settlement of juveniles occurs with gag in northwest Florida seagrass meadows (Koenig and Coleman 1998). Gag spawn offshore on the shelf-edge primarily during February and March (Coleman et al. 1996), and pelagic juveniles settle in shallow seagrass in the spring (Koenig and Coleman 1998, Fitzhugh et al. 2005) during a period of rapid seagrass growth that reaches its peak of biomass and productivity in August (Zieman and Zieman 1989). They egress from the seagrass beds in autumn, moving to offshore reefs during late September to mid-October, when the aboveground biomass and productivity of the seagrass is rapidly declining (Stallings et al. 2010). Thus, juvenile habitat requirements likely contribute to seasonal timing of gag spawning, reminiscent of the pattern observed in goliath grouper. This may be a general pattern among fish species with highly specific settlement habitat requirements.

Determinants of Diel Patterns of Spawning Behavior.—We considered the life history patterns in goliath grouper that represent trade-offs between predation risk and spawning activity to address the question of whether dark-night spawning confers a selective advantage on various life stages of goliath grouper.

Adult goliath grouper spawning on dark nights might fall prey to large sharks feeding at night. It would appear that the abundant low-frequency pulses emitted by spawning groupers (Mann et al. 2009, present study) would attract sharks as several species of shark have been shown to be attracted to pulsed low-frequency sounds (Myrberg 2001), and the distributions of large sharks overlaps the distribution of goliath grouper spawning sites (Graham et al. 2016). However, the limited data available show that large sharks tend to feed during crepuscular times (Hammerschlag et al., this issue) rather than at night. In addition, on all of our dives on goliath grouper spawning aggregations during day and night— hundreds of dives from 1994 through 2015 in the Atlantic Ocean and Gulf of Mexico—we have never observed a non-human-predator-mutilated or injured goliath grouper, although we have observed injuries and partial carcasses from illegal fishing. It would seem with a fish as large as an adult goliath grouper, there would be some physical indication of a natural predation event, but none were observed. We conclude, then, that dark-night spawning is either inconsequential to shark predation or that it confers a selective advantage for reduced risk by temporally separating the activities of predators and spawners.

While it is unlikely that dark-night spawning confers any selective advantage on juveniles settling in the mangroves or on larval survival, it probably is an important factor in minimizing egg predation by the abundant planktivorous fishes present on goliath grouper spawning sites. The planktivorous fishes include round scad, mackerel scad, redtail scad [*Decapterus punctatus* (Cuvier, 1829), *Decapterus macarellus* (Cuvier, 1833), and *Decapterus tabl* Berry, 1968, respectively], and Spanish sardines and round herring [*Sardinella aurita* Valenciennes, 1847 and *Etrumeus teres* (DeKay, 1842)]. Several of these species are known egg predators (Hales 1987, Donaldson and Clavijo 1994). This hypothesis is consistent with the single direct observation of goliath grouper spawning—numerous scad and herring surrounded the presumptive female just prior to the spawning ascent (Fig. 7).

In summary, we used a variety of approaches to evaluate the reproductive locations, timing, and behavior of goliath grouper. These included passive and active acoustics, multiple types of tagging, in situ diver counts made by us and by volunteer divers submitting surveys to REEF, histological analysis of gonad biopsies, and downstream collection of fertilized eggs. All these approaches proved informative, expanding our knowledge of goliath grouper life history and contributing to their conservation. Such approaches would be useful to researchers working in other parts of their range where they remain endangered.

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