TERRITORIALITY, REPRODUCTIVE BEHAVIOR, AND PARENTAL CARE IN GRAY TRIGGERFISH, *BALISTES CAPRISCUS*, FROM THE NORTHERN GULF OF MEXICO

Carrie M. Simmons and Stephen T. Szedlmayer

SEDAR82-RD03

June 14, 2021

BULLETIN OF MARINE SCIENCE. 88(2):197–209. 2012
http://dx.doi.org/10.5343/bms.2011.1012

TERRITORIALITY, REPRODUCTIVE BEHAVIOR, AND PARENTAL CARE IN GRAY TRIGGERFISH, *BALISTES CAPRISCUS,* FROM THE NORTHERN GULF OF MEXICO

Carrie M Simmons and Stephen T Szedlmayer

ABSTRACT

We documented spawning behaviors in gray triggerfish, *Balistes capriscus* (Gmelin, 1789), in June and July 2004–2007 on artificial reefs in the northern Gulf of Mexico, including territoriality, nest building, harem spawning, and parental care. Males were significantly larger than females and could be distinguished from females by size and dark charcoal coloration during the spawning season. Pre-fertilization dominant males were observed building and maintaining one to 13 demersal nests at particular reef sites, aggressively defending the immediate area surrounding the nests against other male gray triggerfish and other fishes, while attracting one to five females to spawn. Pre-fertilization females were observed frequently visiting and inspecting the newly constructed nest. Post-fertilization females stayed continuously on the nest guarding the eggs, and displayed a contrasting white and black color pattern, while fanning and blowing the eggs. Post-fertilization males continued to defend a territory immediately around the nest, visiting the female on the nest, and chasing other fish away. Actual spawning behavior was observed where a male and female tightly circled each other within the nest, with fertilized eggs produced immediately after this circling. Active nests with a guarding female and male were observed 28 times on 18 different reef sites. Mean number of eggs per spawning event was 772,415 from 13 active nests each with a guarding female. Observations of aggressive male behavior and sex ratios of single dominant male and up to five spawning-condition females indicate that gray triggerfish display harem spawning behavior.

Compared to most marine fishes (i.e., pelagic broadcast spawners), triggerfishes (Balistidae) have a combination of atypical spawning behaviors as several species complete some or all of the following behaviors: establish territories, form harem groups, build demersal nests, and display maternal or biparental care of eggs (Fricke 1980, Blumer 1982, Gladstone 1994, Ishihara and Kuwamura 1996, Kuwamura 1997). Previous studies on triggerfish have shown several variations on reproductive behavior, sometimes switching the roles between sexes. For example, the male yellowmargin triggerfish, *Pseudobalistes flavimarginatus* (Ruppell, 1829), migrated to spawning sites, established territories, built demersal nests, and attracted several females to spawn (Gladstone 1994). In contrast, the female yellow-spotted triggerfish, *Pseudobalistes fuscus* (Bloch and Schneider, 1801), built demersal nests and enticed the male into the nest to spawn, while chasing away other courting females, resulting in a 1:1 male to female spawning ratio (Fricke 1980).

Previous studies of reproductive behaviors in triggerfishes have been mainly on Indo-Pacific and Red Sea species (Fricke 1980, Gladstone 1994, Ishihara and Kuwamura 1996, Kuwamura 1997), with little information available on the reproductive behavior of the gray triggerfish, *Balistes capriscus* (Gmelin, 1789), a widely distributed species from both temperate and tropical waters. This species is found

Bulletin of Marine Science

throughout the Gulf of Mexico, the western and eastern Atlantic Ocean, as far north as Nova Scotia, and as far south as Argentina (Briggs 1958, Moore 1967), and is associated with artificial reef structures (Frazer and Lindberg 1994, Vose and Nelson 1994, Kurz 1995, Wilson et al. 1995, Simmons and Szedlmayer 2011) and natural hard-bottom substrate (Johnson and Saloman 1984, Vose and Nelson 1994). In the Gulf of Mexico, the gray triggerfish is an important species to both the commercial and sport fisheries, but is considered to be overfished (SEDAR-9 2006, GMFMC 2008).

Studies have addressed the reproductive biology and seasonality of gray triggerfish spawning: off the coast of Ghana, Africa, gray triggerfish spawning ranged from October to December, but peaked during the warmer months of November and December, and sex ratios were skewed in December with 1 male to 3.2 females (Ofori-Danson 1990). Ofori-Danson (1990) also reported that male gray triggerfish gonads were too small to determine age at first spawning, but 50% of the females were sexually mature at 145 mm fork length (FL) and 100% were mature at 220 mm FL. Wilson et al. (1995) reported that gray triggerfish spawning peaked in June, with a 1:2 male to female sex ratio, and that both sexes were sexually mature by age-2 (250 mm FL) as indicated by histology of commercial landings in the western Gulf of Mexico. A more recent study on gray triggerfish in the Gulf of Mexico found that they were multiple batch spawners, with peak spawning during June and July, and both males and females attaining 100% sexual maturity at 250 mm FL (Ingram 2001). At this size, a few males were age-1, while most were age-2, and all females were age-2 (Ingram 2001). To date, no studies have examined the reproductive behavior of gray triggerfish. Thus, in the present study, we report on the in situ reproductive behavior of gray triggerfish and provide the first description of territoriality, nest building, harem spawning, and parental care in this species.

Methods

The study sites were 26–50 km south to southeast of Dauphin Island, Alabama, in the northern Gulf of Mexico (Fig. 1). Artificial reefs were surveyed because natural reefs are rare in the northern Gulf (Parker et al. 1983, Dufrene 2005). These artificial reefs were located at depths of 20–30 m, which allowed scuba divers adequate time to quantify behaviors of gray triggerfish. A wide variety of artificial reefs were surveyed from 2004 to 2007. In October 2003, 16 artificial reefs were deployed 50 m apart in the Hugh Swingle reef building zone, and consisted of steel cages (2.5 × 1.2 × 1.5 m, Chapin et al. 2009). An additional 44 artificial reefs were deployed 1 km apart in the same area in April and May 2004. During June and July 2004, 45 of these artificial reefs were surveyed for spawning gray triggerfish. In 2005, the search for spawning gray triggerfish was expanded to include May, and we completed 33 surveys on the following reef types: concrete block reefs (1.2 × 1.2 × 0.4 m), a barge (~15 × 6 × 4 m), and gas pipelines (~10 × 2 × 2 m) from 2005 to 2007. In addition, 63 surveys were completed on 18 submerged army tanks (M60, 9.3 × 3.6 × 3.2 m) in June, July, and August 2005, and 82 surveys on 28 army tanks in June and July 2006. In 2007, 62 surveys were completed on 49 reefs in June and July, and spawning gray triggerfish were collected by spearfishing for later sex identification and maturity determination in the laboratory.

Divers recorded the number of all gray triggerfish and videotaped the fish on and around each reef (Sony TR101 Hi-8 video camera). If divers observed demersal nests in the substrate and a gray triggerfish displaying pre-fertilization behaviors, the video camera was placed 1–2 m from the nest to record gray triggerfish behaviors for 30–60 min in the absence of divers. The video camera was placed to observe the nest with the reef in the background, enabling the

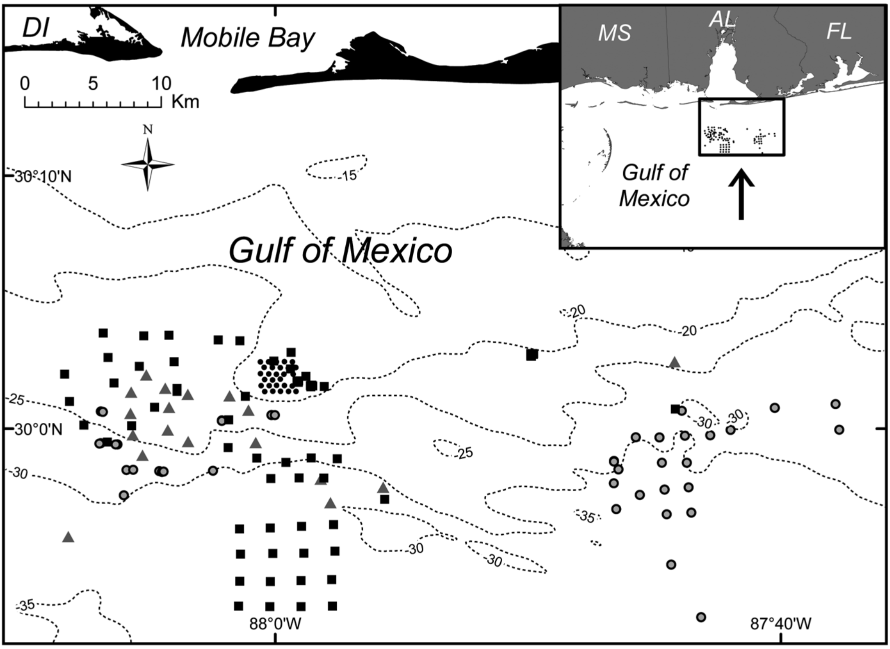

Figure 1. Study location in the northern Gulf of Mexico from 2004 to 2007. Black squares = steel cages, grey triangles = pyramids, grey circles = army tanks, and small black dots = concrete block reefs. Depth contours = m, and DI = Dauphin Island.

recording of the behaviors of gray triggerfish and other fishes on the reef. Not all of the male and female pre- and post-fertilization behaviors could be recorded with the remote video camera. Therefore, when divers surveyed reefs and found gray triggerfish displaying pre- or post-fertilization behaviors, they completed timed surveys and recorded behaviors on dive slates. Also, divers measured the diameter (cm), depth (cm), distance (m) from the reef, and compass bearing to the reef of all nests around each artificial reef. Egg samples (n = 13; all eggs from a nest) and sediment samples (n = 28) were collected with plastic jars (8 × 5 cm) from active nests with a guarding female. Sediment samples were taken from the surface inside the nest where the eggs were attached.

Egg samples were removed from the nest and stored on ice for later laboratory analysis. Prior to preserving the eggs in NOTOXhisto fixative (Scientific Device Laboratory, Glenview, Illinois), 10 eggs from each sample (n = 9) were measured to the nearest 0.01 mm with an Olympus BH-2 compound microscope, Sony AVC-D7 video camera, FlashPoint 128-4M digitizing board (Integral Technologies, Inc., Indianapolis, Indiana), and Image Pro V4.5 software. Different egg masses (n = 13) were weighed to the nearest 0.01 g with a Scout Pro balance (Ohaus, Pine Brook, New Jersey). For egg counts, five 0.054-g samples were taken from each sample and counted under a Leica MZ6 dissecting scope at 10× magnification (Leica Microscopy Systems, Ltd., Heerbrugg, Switzerland) with a Ward Counting Wheel (Wildco, Buffalo, New York). Means of the egg counts were used to estimate number of eggs per nest sample.

Depth, temperature, salinity, and dissolved oxygen were measured with a YSI 6920 meter (Yellow Springs Instruments, Yellow Springs, Ohio) at all spawning sites. Mean values of temperature (n = 21), salinity (n = 21), and dissolved oxygen (n = 16) were derived from all measurements taken within 3 m of the bottom.

Gray triggerfish were collected from 19 army tanks by scuba diver spearfishing for sex ratio determination in August, October, and November 2005, and June–September 2006 (n = 126 gray triggerfish). In June and July 2007, gray triggerfish were collected from an additional 26 artificial reefs for sex ratio determination, and these sample sites included steel cages, concrete pyramids, a barge, concrete block reefs, and a gas pipeline covered with a concrete mat (n = 168 gray triggerfish). From 2004 to 2007, 10 gray triggerfish were collected from various reef types including army tanks, concrete pyramids, steel cages, and concrete block reefs while in the nest guarding eggs. All collected fish were weighed to the nearest 0.01 kg and measured for standard length (SL) and FL to the nearest 1.0 mm. Sex was determined by dissection and gonads were weighed to the nearest 0.01 g.

Substrate samples from active gray triggerfish nests were oven dried at 100–150 °C for 24–72 hrs. Sediment grain size fractions were determined by sorting through 2000, 1000, 500, 250, 106, and 63 μm sieves. Sieve sizes were based on the Wentworth grade limits (Buchanan and Kain 1971). Dried sediment fractions were weighed to the nearest 0.01 g.

Behaviors were divided into pre- and post-fertilization for both male and female gray triggerfish. The number of observations for each behavior was standardized to number per hour, because during diver surveys, different behaviors were recorded as singular events, but were not individually timed over a given period of observation. Male and female pre- and post-fertilization behaviors were compared separately by sex with non-parametric mixed model two-way analysis of variance (ANOVA). The response variable (counts of specific behaviors per hour) failed to meet ANOVA assumptions of normality (Shapiro and Wilk 1965) and equal variances (Brown and Forsythe 1974), therefore an aligned rank transformation method was applied that allowed for testing of interaction effects of two-way ANOVAs (Salter and Fawcett 1993, Higgins and Tashtoush 1994). Sizes (mm SL, FL, and kg) of male to female, dominant males to non-guarding males, nesting to non-nesting females, and specific female ovary weight of nesting to non-nesting fish (ovary wt fish wt^{-1}) during spawning months (June and July) were each compared separately with t-tests (Zar 1999). If t-test comparison groups had significantly different variances, the Satterthwaite correction for unequal variances was applied (Cody and Smith 2006). All tests were considered significant at $\alpha = 0.05$. If significant differences were detected, a Tukey test was used to show specific differences (Zar 1999).

Results

Diver surveys (n = 285) were completed on 186 different artificial reefs. Spawning behaviors were observed in June and July 2004–2007. Active nests with a dominant territorial male and guarding female were observed 28 times, on 18 different reef sites. Gray triggerfish were sexually dimorphic during the spawning season, with males identified by their larger size, darker coloration, black fins, and aggressive behavior toward other fishes and divers. Females displayed a distinct coloration while guarding an active nest, i.e., a sharply contrasting white and black vertical bar pattern across the whole body (Fig. 2). Outside of spawning season, gray triggerfish were only observed with the typical plain gray pigmentation.

All behaviors were recorded during daylight hours 0800 through 1900. Pre-fertilization behavior of gray triggerfish was difficult to document at reef sites because we could not predict if fertilization would follow. However, pre-fertilization behaviors were documented four times, while post-fertilization behaviors were observed 16 times from 2004 through 2006.

Males.—Male gray triggerfish (n = 28) displayed territoriality and patrolled an approximate 15-m diameter area surrounding the nests (n = 154) and reef site. Males appeared to stay and hold particular reef site territories. During the 2006 spawning season, we returned to one particular reef over four separate surveys and found the same dominant male (identity based on a missing section of its caudal fin). Dominant

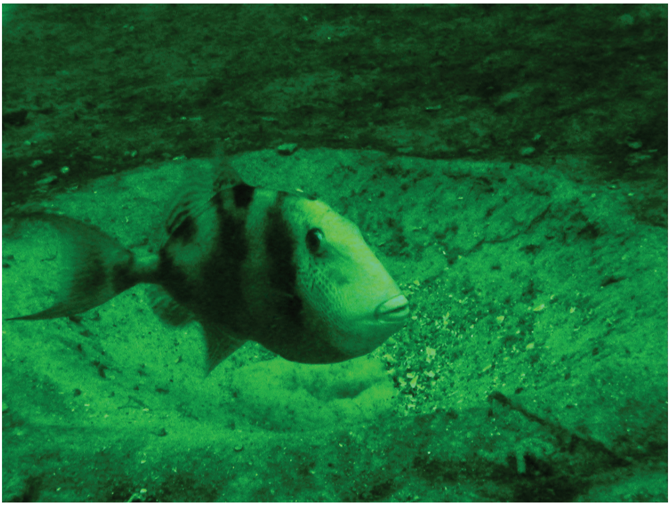

Figure 2. Female gray triggerfish guarding a nest with eggs in the northern Gulf of Mexico.

males were observed chasing other gray triggerfish, red snapper, *Lutjanus campechanus* (Poey, 1860), gag, *Mycteroperca microlepis* (Goode and Bean, 1879), and greater amberjack, *Seriola dumerili* (Risso, 1810), from territories and active nests.

Males were observed guarding up to three active nests at a time. Male pre-fertilization behaviors included nest building and maintenance, chasing fish, and circling in the nest with a female. Male post-fertilization behaviors included chasing fish and nest visits. For males, significant differences were detected among pre- and post-fertilization time periods (ANOVA: $F_{1, 67} = 14.36$, $P < 0.001$), behavior types ($F_{3, 67} = 7.78$, $P < 0.001$), and behavior-time interaction ($F_{3, 67} = 10.7$, $P < 0.001$). Nest building and maintenance during pre-fertilization was only observed in males and significantly more (mean = 33.3 hr^{-1}) than post-fertilization nest building and maintenance and most other male pre- and post-fertilization behaviors (chasing fish, circling in the nest with a female, and nest visits). There were no significant differences detected among these other male pre- and post-fertilization behaviors (Fig. 3). A new behavior observed in post-fertilization males (not observed pre-fertilization) was visiting the female gray triggerfish on the nest (16.1 hr^{-1}, Fig. 3).

Females.—Females generally stayed on or near the reef structure during the spawning season, except immediately after fertilization at which time they remained almost continuously on the nest. Three pre-fertilization behaviors were observed for female gray triggerfish including nest inspection, foraging, and female nest competition, and three post-fertilization behaviors were observed including foraging, blowing and fanning eggs, and chasing fish. For females, significant differences were detected among times (ANOVA: $F_{1, 99} = 54.4$, $P < 0.001$), behaviors ($F_{4, 99} = 27.3$, $P < 0.001$), and behavior-time interaction ($F_{4, 99} = 14.5$, $P < 0.001$). Only females were observed blowing and fanning the eggs post-fertilization, and this behavior was observed significantly more frequently (mean = 106.8 hr^{-1}) than any of the other behaviors (Fig. 4). Females were often observed chasing other fishes (mean 25.6 hr^{-1}), but only during post-fertilization periods, although no significant differences were detected (Fig. 4). However, competitive interactions between two females were observed pre-fertilization. In this interaction, two females briefly circled each other in

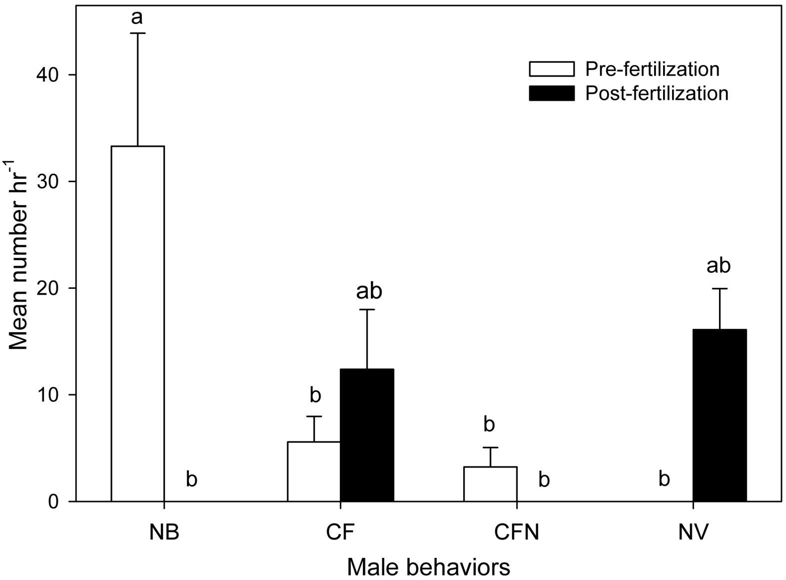

Figure 3. Pre- (white bars, n = 3) and post-fertilization (black bars, n = 14) behaviors of male gray triggerfish in the northern Gulf of Mexico from 2004 to 2007. Behaviors: NB = nest building and maintenance, CF = chasing fishes, CFN = circling with female in nest, and NV = nest visits. Error bars = standard error. Significant differences ($\alpha = 0.05$) are indicated by different letters.

a nest, with one female eventually chasing the other from the nest. Post-fertilization, female gray triggerfish were recorded chasing potential egg predators from the following families: Lutjanidae, Labridae, Haemulidae, and Serranidae. Chasing patterns of females differed from males as they only made short attacks (< 2 m) toward other fishes before quickly returning to the nest. On a few occasions, females were also observed aggressively swimming toward the dominant guarding male, but quickly retreated back to the nest after apparent recognition of the dominant male.

A post-fertilization behavior observed only in female gray triggerfish was their diligence in staying on the nest with the eggs. Females were rarely observed leaving the nest in response to scuba divers, competition, or potential predators. One exception was recorded via the remote video camera, when a female quickly left the nest and moved to the shelter of the artificial reef (army tank) when a 2-m sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827), passed directly over the nest. After the shark passed, she quickly returned to the nest. Females on the nest were occasionally observed searching for food by blowing in the sand within 1 m around the nest, but they were never observed consuming food in either the pre- or post-fertilization period (Fig. 4).

Spawning.—The circling of a male and female in a nest with rapidly changing body colors was considered the last behavioral step before spawning and was observed twice (Fig. 3). Gamete release and fertilization occurred shortly (< 30 min) after based on subsequent scuba diver observations of a guarding female on the nest with eggs. This guarding female on the nest was not present prior to the circling behaviors.

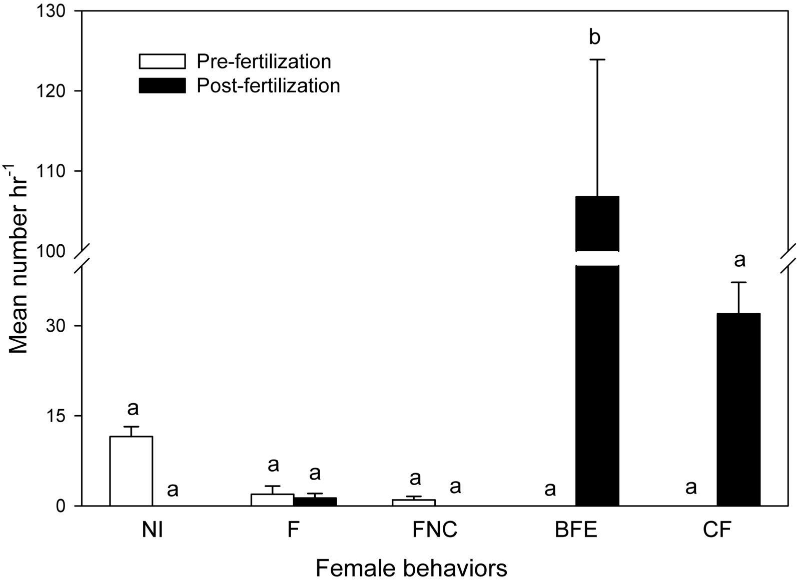

Figure 4. Pre- (white bars, n = 4) and post-fertilization (black bars, n = 16) behaviors of female gray triggerfish in the northern Gulf of Mexico from 2004 to 2007. Behaviors: NI = nest inspection, F = foraging; FNC = female nest competition, BFE = blowing and fanning eggs, and CF = chasing fishes. Error bars = standard error. Significant differences (α = 0.05) are indicated by different letters.

Nest Parameters.—Based on compass bearings, nests were distributed around the reef without any noticeable pattern. The maximum number of nests counted around a reef was 13, with a mean ± SE = 4 ± 0.02 per reef during the spawning season. Mean ± SE nest diameter = 53.1 ± 1.3 cm, depth = 23.6 ± 0.6 cm, and distance from the reef = 8.7 ± 0.4 m (n = 154). Nest substrate mainly consisted of fine sand (250 µm, 67%), followed by coarse sand (1000 µm, 10%), medium sand (500 µm, 9.6%), coarse shell (2000 µm, 9%), and very fine sand and silt (106 and 63 µm, 4%, n = 28). Eggs were deposited in a single cohesive mass at the bottom of the nest. A gelatinous protein matrix held the egg mass together. Eggs were negatively buoyant, even when separated from the egg mass. Pieces of shell and sediment were attached to the bottom of the egg mass. Mean ± SE egg diameter was 0.62 ± 0.003 mm (n = 90) based on samples from nine different nests, and mean ± SE eggs per nest was 772,415 ± 20,545 eggs (range: 420,486–1,371,409 eggs, n = 13). Eggs were observed hatching between 24 and 48 hrs after fertilization based on samples returned to the laboratory. Most egg masses successfully hatched in the laboratory. One exception was the last egg mass collected in late July where eggs failed to hatch, when the water temperature was at the upper limit (29.9 °C) for gray triggerfish spawning.

Gray triggerfish spawning was observed at a mean ± SE depth of 23.7 ± 0.2 m (range: 14.6–31.1 m, n = 21), temperature = 23.4 ± 0.1 °C (range: 21.9–29.9 °C, n = 21), salinity = 34.6 ± 0.1 (range: 29.8–36.8, n = 21), and dissolved oxygen concentration = 5.7 ± 0.7 mg L^{-1} (range: 4.8–6.8 mg L^{-1}, n = 16).

Fish Collections.—When collecting gray triggerfish, divers identified what they assumed were males based on darker color pattern, aggression toward divers, and

Table 1. Sex ratios of all gray triggerfish on artificial reefs in the northern Gulf of Mexico when an active nest and guarding male were observed. Asterisk indicates that all gray triggerfish were not collected from that reef. Two fish were missed from a concrete pyramid (8) and one fish was missed from each concrete block reef (9 and 10).

Reef number	Reef type	Reef size (m)	Males	Females
1	Army tank	9.3 × 3.6 × 3.2	1	4
2	Concrete pyramid	3.7 × 3.7 × 3.1	1	4
3	Steel cage	2.5 × 1.2 × 1.5	1	3
4	Steel cage	2.5 × 1.2 × 1.5	1	5
5	Concrete pyramid	3.7 × 3.7 × 3.1	1	5
6	Army tank	9.3 × 3.6 × 3.2	1	1
7	Army tank	9.3 × 3.6 × 3.2	2	4
8	Concrete pyramid	3.7 × 3.7 × 3.1	10	8*
9	Concrete block reef	1.2 × 1.2 × 0.4	3	2*
10	Concrete block reef	1.2 × 1.2 × 0.4	0	2*

larger size, and collected these fish first. Dominant male identification based on this color pattern and aggressive behavior was 100% accurate (n = 8). Males were significantly larger (mean ± SE: 0.57 ± 0.03 kg, 226.3 ± 3.6 mm SL, 281.7 ± 4.3 mm FL, n = 139) than females (0.46 ± 0.02 kg, 210.7 ± 2.7 mm SL, 262.4 ± 3.2 mm FL, n = 156; SL t-test: $t_{262} = 3.45$, $P < 0.001$). Mean male to female sex ratios at sites without spawning (lack of active nests) was 1:1.3 (n = 49 reefs). Harems (one male and several females) were observed 50% of the time at sites with active nests, and showed a 1:4.2 ration (Table 1). Most gray triggerfish females that were collected from a particular reef site during the spawning season of June and July showed a similar maturity level based on gonad weight (i.e., only four immature fish were collected compared to 97 mature female fish from reef sites). There was no significant difference in specific gonad weight (gonad g to fish weight g^{-1}) between nesting (mean ± SE = 0.038 ± 0.006, n = 10) and non-nesting females (0.046 ± 0.003, n = 87; t-test: $t_{95} = 0.86$, $P = 0.39$). The similarity of reproductive state for nesting and non-nesting females provides evidence that most (97 out of 101) females on a particular reef were in spawning condition and would imminently spawn with the attendant dominant male.

All fish removed from active nests were females. Size of females on active nests in June and July (0.44 ± 0.06 kg, 210.1 ± 10.7 mm SL, 260.2 ± 11.7 mm FL, n = 10) were not significantly different from other females on reef sites (0.45 ± 0.02 kg, 209.6 ± 3.4 mm SL, 260.8 ± 4.0 mm FL, n = 110; SL t-test : $t_{118} = 0.04$, $P = 0.97$). Territorial dominant guarding males in June and July were significantly larger (0.99 ± 0.22 kg, 274.4 ± 18.5 mm SL, 337.3 ± 23.0 mm FL, n = 8) than other males (0.52 ± 0.03 kg, 220.7 ± 4.1 mm SL, 274.9 ± 4.9 mm FL, n = 103; SL t-test: $t_{109} = 3.46$, $P < 0.001$).

Discussion

The present observations on the reproductive behavior of gray triggerfish are consistent with past studies that showed spawning peaks during June and July, and that males are larger than females (Wilson et al. 1995, Ingram 2001, Moore 2001). Other triggerfish species have also shown sex-related size differences including: red-toothed triggerfish, *Odonus niger* (Ruppell, 1836), white-banded triggerfish, *Rhinecanthus aculeatus* (Linnaeus, 1758), halfmoon triggerfish, *Sufflamen chrysopterum* (Bloch and Schneider, 1801), and yellow-spotted triggerfish (Fricke 1980, Ishihara and Kuwamura 1996, Kuwamura 1997).

In addition to size differences, dominant male gray triggerfish could be identified by coloration, territorial behavior, and the construction and maintenance of several nests. Similarly, male yellowmargin triggerfish maintain nests and showed fish-chasing behaviors (Gladstone 1994), and male halfmoon triggerfish also defend territories prior to spawning (Kawase and Nakazono 1992). Gray triggerfish build smaller nests than these other two species of Balistidae, likely due to the general smaller size of this species (Lobel and Johannes 1980, Gladstone 1994). A behavior not previously reported in Balistidae is the male and female gray triggerfish circling tightly in the nest and rapidly changing colors. A similar behavior to circling in the nest was recorded for the male halfmoon triggerfish, termed "lateral display," prior to fertilization of the eggs. However, the female halfmoon triggerfish showed a different behavior, termed "dipping," and both fish were outside the nest when these behaviors were observed (Ishihara and Kuwamura 1996).

Pre-fertilization female gray triggerfish were often observed visiting and inspecting nests, as was shown for female yellowmargin triggerfish (Gladstone 1994). Female gray triggerfish were rarely observed foraging. In contrast, the halfmoon triggerfish commonly foraged prior to spawning (Ishihara and Kuwamura 1996). Another unreported behavior in previous Balistidae studies was two female gray triggerfish circling in the nest. This display may have been a mechanism to identify the sex of the other fish or perhaps establish dominance. Although female yellow-spotted triggerfish show same-sex competition for spawning access to a territorial male, Fricke (1980) did not report females circling in the nest with another female. Most other species of Balistidae do not show aggressive behavior among females; however, past studies have been based entirely on diver observations while our study incorporated remote video recording to examine behaviors over longer time periods.

There are few estimates of the number of eggs per egg mass for any species of triggerfish. Lobel and Johannes (1980) estimated 430,000 eggs per nest (n = 1) for yellowmargin triggerfish, and Kawase and Nakazono (1992) estimated that halfmoon triggerfish produced 132,800 eggs per nest (n = 2). Both of these previous estimates are lower than our egg number estimates for gray triggerfish (mean number = 772,415 eggs for mean fish size = 261 mm FL). Ingram (2001) also showed high fecundity for gray triggerfish ranging from 96,379 to 2,649,027 oocytes for mean fish size of 326 mm FL.

Both male and female triggerfish showed aggressive behavior toward conspecifics and potential egg predators. Both chased other fishes away from the nest, but the male patrolled a wider area surrounding the nest while the female always returned quickly to the nest. Female behaviors were clearly related to "parental care," e.g., blowing and fanning the egg mass, but it is more difficult to ascribe parental care to males as separate from territorial defense or protection of the female. Similar behaviors are showed by the male red-toothed and yellowmargin triggerfishes (Fricke 1980, Gladstone 1994).

All gray triggerfish collected within nests were females and they rarely moved off the nest. In other triggerfish species, females are also the primary fish within the nest (Fricke 1980, Kawase and Nakazono 1992, Gladstone 1994, Ishihara and Kuwamura 1996, Kuwamura 1997). The red-toothed triggerfish was not as diligent about guarding the nest, often leaving eggs and swimming up in the water column for short periods, and visiting her sleeping site during the day (Fricke 1980). Post-fertilization behaviors of the female halfmoon triggerfish are similar to the female gray triggerfish except that they forage more often and farther from the nest (Kawase and Nakazono 1992). Similar to gray triggerfish, the halfmoon triggerfish and yellow-spotted triggerfish also blow and fan the egg mass, and these behaviors were suggested as a means for dispersion of the larvae (Fricke 1980, Kawase and Nakazono 1992).

Gray triggerfish were observed spawning at temperatures ranging from 21.9 to 29.9 °C; however, the maximum of 29.9 °C occurred in late July at the end of the spawning season and the eggs failed to hatch. Little information is available on range of spawning temperatures and subsequent hatching success of the eggs for other species of triggerfish, but the following temperatures were documented when spawning occurred: 24–27.8 °C for masked triggerfish, *Sufflamen fraenatum* (Latreille, 1804), and yellow-spotted triggerfish (Fricke 1980, Kawabe 1984), and 26.6–28.8 °C for halfmoon triggerfish during the month of August (Ishihara and Kuwamura 1996).

Gray triggerfish spawned at greater depths (14.6–31.1 m) compared to most other triggerfish with reported spawning depths of 1–13 m (Fricke 1980, Lobel and Johannes 1980, Kawase and Nakazono 1992, Gladstone 1994, Ishihara and Kuwamura 1996, Kuwamura 1997). Gray triggerfish could potentially spawn at both shallower and deeper depths than we observed, but we did not sample reef sites at these depths.

Gray triggerfish nest sites contained more fine sand (250 μm) than the other sediment size fractions, and attached coarse shell to the egg mass. Other triggerfish species, i.e., halfmoon, orange-lined, *Balistapus undulatus* (Park, 1797), and red-toothed, also have a preference for sand substrate and some mixed the eggs with sand (Fricke 1980, Lobel and Johannes 1980, Kawase and Nakazono 1992, Ishihara and Kuwamura 1996). Yellowmargin triggerfish build nests in sand sediments, but also lined the nests with pieces of coral rubble (Gladstone 1994) and attached some coral to the egg masses (Lobel and Johannes 1980).

When active gray triggerfish nests were found, harems were observed 50% of the time. Similarly, most species of triggerfish have shown harem spawning behavior, with some exceptions: for example, male yellow-spotted triggerfish mate sequentially with one female at a time (Fricke 1980). There were several potential reasons why we only observed harems 50% of the time during the spawning season. Anecdotal observations suggest that one reason may be that occasionally guarding males are unable to exclude all other males from the reef. Another scenario may be that competition and aggression are actively occurring, but the end result of a defined harem had not yet occurred at the time of sampling. Lastly, formations of harems in gray triggerfish may be variable and not always occur. For example, Fricke (1980) suggested that whether a fish is monogamous (one male mates with one female in a spawning season) or polygynous (one male fertilizes the eggs of several females in a spawning season) was based on environmental factors, the establishment of a territory, and attraction of enough mates. Ishihara and Kuwamura (1996) also found that halfmoon triggerfish can be monogamous or polygynous based on the size of the male and location of their territory.

One unknown aspect of gray triggerfish spawning behavior is whether fish return to particular reef sites for spawning. Other species of triggerfish including the red-toothed triggerfish and yellowmargin triggerfish move to particular spawning grounds (Fricke 1980, Gladstone 1994). In our study, we observed the same dominant male (with recognizable scars and damaged fins) at one reef site on four different surveys, and its continued presence along with new females suggested that harem spawning behaviors were related to specific spawning grounds as observed in other triggerfishes (Fricke 1980, Gladstone 1994).

Egg size of gray triggerfish was small (mean = 0.62 mm), similar to other species of triggerfish including the masked triggerfish, halfmoon triggerfish, yellowmargin triggerfish, and orange-lined triggerfish (Lobel and Johannes 1980, Kawabe 1984, Kawase and Nakazono 1992, Gladstone 1994). Parental care typically is not observed in species with small egg sizes (Shine 1978, Gross and Sargent 1985, Sargent et al. 1987, Nussbaum and Schultz 1989). One possible explanation for our observations of increased parental care with small egg sizes may be due to environmental limits (Shine 1978, Nussbaum and Shultz 1989). For example, small eggs with a high surface area to volume ratio would be more suited to a low oxygen environment that might be expected in small depressions at the sediment water interface on the deeper continental shelf habitats. This contention is supported by observations of female gray triggerfish repeatedly fanning the egg masses. Similarly, in a study of the green bubble goby, *Eviota prasina* (Klunzinger, 1871), the time male fish spent fanning eggs was the most important factor affecting survival of eggs, and fanning occurred at a higher frequency for larger egg masses (Karino and Arai 2006).

In summary, in the northern Gulf of Mexico male gray triggerfish establish territories and dominance on artificial reefs and build demersal nests in the sand to attract females to spawn. Females inspect the nests and circle in the nest with the male prior to release of gametes. Gray triggerfish display parental care, with the female fanning and blowing on the eggs, and only swimming off the nest to briefly chase potential egg predators away. Gray triggerfish form harems, which is consistent with other species in the family Balistidae. These observations of elaborate pre-fertilization behavior, parental care, and territory defense are frequently observed in coral reef fishes (Mumby and Wabnitz 2002), but are unusual for marine fish species found on artificial reefs in the northern Gulf of Mexico and provide new information on the reproductive ecology of marine fishes in this system. This new information on gray triggerfish spawning behavior may have management implications: e.g., the removal of the aggressive male from the reef may disproportionally reduce spawning success of several females.

Acknowledgments

We thank S Beyer, A Chapin, L Grove, D Miller, D Nadeau, R Redman, D Topping, R Wingate, and B Wildberger for field assistance. This project was funded by the Mississippi-Alabama Sea Grant Consortium (MASGC R/SP-14) NOAA award number NA06OAR4170078 and Marine Resources Division, Alabama Department of Conservation and Natural Resources. This study is a contribution of the Alabama Agricultural Experiment Station and Department of Fisheries and Allied Aquacultures, Auburn University.

Literature Cited

Blumer LS. 1982. A bibliography and categorization of bony fishes exhibiting parental care. Zool J Linn Soc. 75:1–22. http://dx.doi.org/10.1111/j.1096-3642.1982.tb01939.x

Briggs JC. 1958. A list of Florida fishes and their distribution. Bull Fla State Mus Biol Sci. 2:223–318.

Brown MB, Forsythe AB. 1974. Robust tests for equality of variances. J Am Stat Assoc. 69:364–367. http://dx.doi.org/10.2307/2285659

Buchanan JB, Kain JM. 1971. Measurement of the physical and chemical environment. *In:* Holme NA, McIntyre AD, editors. Methods for the study of marine benthos. 1st ed. Oxford and Edinburgh: Blackwell. p. 30–58.

Chapin AM, Szedlmayer ST, Phelps RP. 2009. Survival and movement of hatchery-reared red snapper on artificial reefs in the northern Gulf of Mexico. Fish Manage Ecol. 16:28–36. http://dx.doi.org/10.1111/j.1365-2400.2008.00620.x

Cody RP, Smith JK. 2006. Applied statistics and the SAS programming language. 5th ed. Upper Saddle River, Pearson Prentice Hall, Inc.

Dufrene TA. 2005. Geological variability and Holocene sedimentary record on the northern Gulf of Mexico inner to mid-continental shelf. MS Thesis. Louisiana State Univ, Baton Rouge. 100 p.

Frazer TK, Lindberg WJ. 1994. Refuge spacing similarly affects reef-associated species from three phyla. Bull Mar Sci. 55:388–400.

Fricke HW. 1980. Mating systems, maternal and biparental care in triggerfish (Balistidae). Z Tierpsychol. 53:105–122. http://dx.doi.org/10.1111/j.1439-0310.1980.tb01043.x

Gladstone W. 1994. Lek-like spawning, parental care and mating periodicity of the triggerfish *Pseudobalistes flavimarginatus* (Balistidae). Environ Biol Fish. 39:249–257. http://dx.doi.org/10.1007/BF00005127

GMFMC (Gulf of Mexico Fishery Management Council). 2008. Reef Fish Amendment 30A: greater amberjack-revised rebuilding plan, accountability measures and gray triggerfish-establish rebuilding plan, end overfishing, accountability measures, regional management, management thresholds and benchmarks, Tampa, Florida.

Gross MR, Sargent RC. 1985. The evolution of male and female parental care in fishes. Am Zool. 25:807–822.

Higgins JJ, Tashtoush S. 1994. An aligned rank transform test for interaction. Nonlinear World. 1:201–211.

Ingram GW Jr. 2001. Stock structure of gray triggerfish, *Balistes capriscus,* on multiple spatial scales in the Gulf of Mexico. PhD dissertation, Univ of South Alabama, Mobile. 228 p.

Ishihara M, Kuwamura T. 1996. Bigamy or monogamy with maternal egg care in the triggerfish, *Sufflamen chrysopterus*. Ichthyol Res. 43:307–313. http://dx.doi.org/10.1007/BF02347602

Johnson AG, Saloman CH. 1984. Age, growth, and mortality of gray triggerfish, *Balistes capriscus,* from the northeastern Gulf of Mexico. Fish Bull. 82:485–492.

Karino K, Arai R. 2006. Effect of clutch size on male egg-fanning behavior and hatching success in the goby, *Eviota prasina* (Klunzinger). J Exp Mar Biol Ecol. 334:43–50. http://dx.doi.org/10.1016/j.jembe.2006.01.018

Kawabe R. 1984. Spawning behavior of the bridled triggerfish, *Sufflamen fraenatus,* in the aquarium. Japan J Ichthyol. 31:193–197.

Kawase H, Nakazono A. 1992. Reproductive Behavior of the flagtail triggerfish, *Sufflamen chrysopterus.* Proc 7th Int Coral Reef Symp. 2:905–907.

Kurz RC. 1995. Predator-prey interactions between gray triggerfish (*Balistes capriscus* Gmelin) and a guild of sand dollars around artificial reefs in the northeastern Gulf of Mexico. Bull Mar Sci. 56:150–160.

Kuwamura T. 1997. Evolution of female egg care in haremic triggerfish, *Rhinecanthus aculeatus.* Ethology. 103:1015–1023. http://dx.doi.org/10.1111/j.1439-0310.1997.tb00143.x

Lobel PS, Johannes RE. 1980. Nesting, eggs and larvae of triggerfishes (Balistidae). Environ Biol Fish. 5:251–252. http://dx.doi.org/10.1007/BF00005359

Moore D. 1967. Triggerfishes (Balistidae) of the western Atlantic. Bull Mar Sci. 17:689–722.

Moore JL. 2001. Age, growth and reproductive biology of the gray triggerfish (*Balistes capriscus*) from the southeastern United States, 1992–1997. MS thesis, Univ of Charleston, Charleston. 69 p.

Mumby PJ, Wabnitz CCC. 2002. Spatial patterns of aggression, territory size, and harem size in five sympatric Caribbean parrotfish species. Environ Biol Fish. 63:265–279. http://dx.doi.org/10.1023/A:1014359403167

Nussbaum RA, Schultz DL. 1989. Coevolution of parental care and egg size. Am Nat. 133:591–603. http://dx.doi.org/10.1086/284939

Ofori-Danson PK. 1990. Reproductive ecology of the triggerfish, *Balistes capriscus* from the Ghanaian coastal waters. Trop Ecol. 31:1–11.

Parker RO, Colby DR, Willis TD. 1983. Estimated amount of reef habitat on a portion of the US South Atlantic and Gulf of Mexico continental shelf. Bull Mar Sci. 33:935–940.

Salter KC, Fawcett RF. 1993. The art test of interaction: a robust and powerful rank test of interaction in factorial models. Comm Stat Simulat. 22:137–153. http://dx.doi.org/10.1080/03610919308813085

Sargent RC, Taylor PD, Gross MR. 1987. Parental care and the evolution of egg size in fishes. Am Nat. 129:32–46. http://dx.doi.org/10.1086/284621

SEDAR-9 (Southeast data assessment and review). 2006. Stock assessment report 1. Gulf of Mexico gray triggerfish. Charleston: Southeast Fisheries Science Center. (http://www.sefsc.noaa.gov/sedar).

Shapiro SS, Wilk MB. 1965. An analysis of variance test for normality (complete samples). Biometrika. 52:591–611.

Shine R. 1978. Propagule size and parental care. The "safe harbor" hypothesis. J Theor Biol. 75:417–424. http://dx.doi.org/10.1016/0022-5193(78)90353-3

Simmons CM, Szedlmayer ST. 2011. Recruitment of age-0 gray triggerfish to benthic structured habitat in the Northern Gulf of Mexico. Trans Am Fish Soc. 140:14–20.

Vose FE, Nelson WG. 1994. Gray triggerfish (*Balistes capriscus* Gmelin) feeding from artificial and natural substrate in shallow Atlantic waters of Florida. Bull Mar Sci. 55:1316–1323.

Wilson CA, Nieland DL, Stanley AL. 1995. Age, growth, and reproductive biology of gray triggerfish, *Balistes capriscus,* from the Northern Gulf of Mexico commercial harvest. MARFIN Final Report. Baton Rouge: Louisiana State Univ.

Zar JH. 1999. Biostatistical Analysis. 4th ed. Upper Saddle River: Prentice-Hall, Inc.

Date Submitted: 8 February, 2011.
Date Accepted: 18 November, 2011.
Available Online: 22 December, 2011.

Addresses: (CMS) *Gulf of Mexico Fishery Management Council, 2203 North Lois Avenue, Suite 1100, Tampa, Florida 33607.* (STS) *Department of Fisheries and Allied Aquacultures, Auburn University, 8300 State Highway 104, Fairhope, Alabama 36532.* Corresponding Author: (STS) *E-mail: <szedlst@auburn.edu>.*