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ARTICLE

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Abstract

Effective fisheries management requires a detailed understanding of the life history strategies of managed species. The Queen Triggerfish *Balistes vetula* supports productive fisheries in the western Atlantic, including the U.S. Caribbean. We utilized a combination of fishery-dependent and fishery-independent samples to assess the size structure, sex ratio, size at maturity, spawning season, and spawning frequency for a Queen Triggerfish population in the U.S. Caribbean. From 2013 to 2018, 1,148 samples were collected, ranging in size from 67 to 434 mm FL. This study provides important life history information from an exploited population and is the first to describe Queen Triggerfish reproductive biology in detail for the Caribbean. We documented that the Queen Triggerfish is a sexually dimorphic species characterized by a medium size at maturity. The smallest sexually mature male and female were 184 and 215 mm FL, respectively. Lengths at 50% sexual maturity (L_{50}) for males sampled from Puerto Rico and St. Croix, U.S. Virgin Islands, were similar (206 and 211 mm FL, respectively) and were significantly smaller than the L_{50} values for females (Puerto Rico: 256 mm FL; St. Croix: 245 mm FL). Queen Triggerfish, nesting benthic spawners, exhibited group-synchronous oogenesis and indeterminate fecundity over the spawning season that started as early as

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the week after the full moon in December and extended until August. Spawning interval, defined as the number of days between spawning events in a female, was 54–55 d, indicating that a female could spawn up to five times over the estimated 241–267-d spawning season. As regulations on grouper and snapper species in the Caribbean increase, Queen Triggerfish will experience increasing fishing pressure. Managers should continue to evaluate potential impacts of this pressure and establish management regulations that consider the region-specific reproductive season and size at maturity.

Effective fisheries management requires a detailed understanding of the life history strategies of managed species (Chale-Matsau et al. 2001; King and McFarlane 2003). Triggerfish species (family Balistidae) occur in temperate and tropical waters of the Atlantic, Pacific, and Indian oceans (Matsuura 2015). Species from the genus *Balistes* support important fisheries in the Atlantic Ocean (Gray Triggerfish *B. capriscus* and Queen Triggerfish *B. vetula*); north and south of the equator (Bernardes 2002; Barroso-Soto et al. 2007; NMFS 2009; Aggrey-Fynn and Sackey-Mensah 2012); in the Mediterranean Sea (Kacem and Neifar 2014; Kacem et al. 2015), Gulf of Mexico (SEDAR 2006), and Caribbean Sea (Matos-Caraballo 2012; SEDAR 2013); and in the Pacific Ocean (Finescale Triggerfish *B. polylepis*; eastern Pacific). However, little published information exists on the reproductive biology of several *Balistes* species.

Queen Triggerfish is a moderately long-lived (Albuquerque et al. 2011) and moderately large benthic reef fish adapted to slow movements, foraging on hard-shelled invertebrates off and around reef structures (Durie and Turingan 2001). Predation on Queen Triggerfish and other triggerfish is minimized due to tough skin and a large dorsal spine that is only retracted when the next small spine is depressed or "triggered" (Aiken 1983). Queen Triggerfish are distributed in the western Atlantic from as far north as North Carolina to as far south as Brazil (Ferreira Menezes 1979; Parker and Dixon 1998). The distribution and habitat preferences of the Queen Triggerfish in many ways are similar to those of its congener, Gray Triggerfish, which occurs around hard-bottom habitats (Sedberry and Van Dolah 1984), along rocky outcrops and ridges (Gledhill 2005), and in association with offshore oil rigs to depths of 61 m (Stanley and Wilson 2003). One study (Randall 1963) reported that Queen Triggerfish in the size range of 215–330 mm FL fed on sea urchins during daylight hours at an artificial reef site in the U.S. Virgin Islands (USVI).

Manooch and Drennon (1987) examined age and growth of Queen Triggerfish in Puerto Rico (PR) and the USVI. They used dorsal spines for aging specimens collected in 1983–1984 and reported that annulus formation occurred from February to March and that the maximum age was 7 years. Ferreira Menezes (1979) examined age and growth in Queen Triggerfish from Brazilian waters and reported a maximum age of 9 years. A more recent study on age and growth of Queen Triggerfish from St. Thomas, USVI, reported a maximum age of 14 years (V. Shervette, unpublished data).

Triggerfish species, in general, exhibit a relatively unusual mating strategy compared to other fisheries-targeted reef fish species (Lobel and Johannes 1980; Gladstone 1994; Kuwamura 1997; Kawase 2003; Simmons and Szedlmayer 2012). Many balistid species are characterized by lek-like spawning systems in which a male establishes and defends a nesting territory, the males and females construct benthic nests for the individual females to lay their eggs in, and the adults guard nests and care for the developing embryos after fertilization until the larvae emerge. Simmons and Szedlmayer (2012) investigated this reproductive strategy in the closely related congener, the Gray Triggerfish, and documented that an individual female stays inside the nest and guards the eggs while the male guards the territory surrounding the nest. These behaviors continue until the larvae emerge, which occurs within 24–48 h after fertilization (Simmons and Szedlmayer 2012).

Little information exists concerning the reproductive biology and ecology of Queen Triggerfish in U.S. waters. Ferreira Menezes (1979) reported that Queen Triggerfish spawned in Brazilian waters mainly during March and April and that the size range of sexually mature individuals was 238–502 mm FL. Anecdotal information from spear-fishers in St. Croix (STX), USVI, indicates that Queen Triggerfish establish and guard nests starting as early as December during the week after the full moon (Shervette, unpublished data; G. Martinez, STX spearfisher, personal communication).

In many ways, PR and USVI share similar histories of the evolution of their reef fishing industries. Currently, both regions have commercial fisheries described using terms such as "artisanal," "subsistence," and "small-scale," meaning that they support internal seafood needs, with only a small portion (if at all, depending on the species) of catches sold outside of the islands. Additionally, U.S. waters in the Caribbean attract recreational anglers and divers from around the world, which supplies tourism dollars to the local economies. Commercial and recreational fisheries target Queen Triggerfish in the U.S. Caribbean (Bryan 2012; McCarthy 2012), and it is one of

the top commercial demersal reef fishery species in U.S. Caribbean waters. In USVI, it ranks third in reconstructed annual landings from 1950 to 2010 for commercial reef fish (Ramdeen et al. 2015); in PR, it has consistently remained one of the top-seven targeted reef fish species over the past 20 years in terms of kilograms landed (Matos-Caraballo 2007, 2012). Annual commercial landings for the region in 2000–2011, summarized by McCarthy (2012), were obtained from fisher logbook reports. Queen Triggerfish landings in PR have been variable since 2000 but generally trended around 27,216–31,751 kg (60,000–70,000 lb; McCarthy 2012). During the same period in USVI (St. Thomas, St. John, and STX), annual commercial landings for "triggerfishes" increased from 43,048 kg (94,905 lb) to a maximum of 59,624 kg (131,449 lb) in 2002 and then declined to 25,329 kg (55,841 lb) in 2011 (McCarthy 2012).

Queen Triggerfish is a data-deficient species due to the lack of species-specific biological data in the U.S. Caribbean. The most recent stock assessment for Queen Triggerfish from U.S. waters concluded that the lack of current species-specific life history information greatly hindered the assessment and, given the data limitations, the projections for future status of the stock could not be constructed (SEDAR 2013). The main goal of the current study was to fill in the critical information gaps concerning Queen Triggerfish reproductive biology in U.S. waters and the Caribbean in general. Our specific objectives were to determine and compare the following for Queen Triggerfish in PR and USVI waters: (1) size structure and sex ratio; (2) size at sexual maturity; (3) spawning seasonality and frequency; and (4) oocyte development type and fecundity type.

METHODS

Fish collection and processing.— Our study occurred within two main areas of the Caribbean: the south and west coasts of PR; and the coastal areas surrounding STX, USVI. We collected Queen Triggerfish samples from a combination of fishery-dependent and fishery-independent sources (Table 1). Monthly fishery-dependent samples were purchased from PR fishers between July 2013 and March 2018 and from STX fishers between December 2015 and March 2018. In PR and STX, spear-fishers were instructed to land fish of all sizes that were big enough to spear, and trap fishers were instructed to retain all fish so that we could intercept them as they returned from fishing and purchase their catch at the landing. Fishery-independent samples were collected opportunistically by hook-and-line fishing and spear-fishing. All samples were kept on ice until initial processing occurred. For each fish sample, we measured SL, FL, and TL to the nearest millimeter and total weight to the nearest gram. Gonads were removed, weighed whole to the nearest 0.01 g, and then preserved for later histological processing to determine sex, sexual maturity, and reproductive phase using criteria modified from Kelly-Stormer et al. (2017; Table 2).

To determine whether mean size significantly differed between sexes and islands, we used a two-factor ANOVA with size (FL) as the dependent factor and with island and sex as the independent factors. The relationship between FL and weight was assessed using separate linear regressions for each island × sex combination. Weight data were square-root transformed to meet the assumption of normality. To determine whether this relationship differed significantly by island and sex, we used an ANCOVA with weight as the dependent variable, length as the covariate, and island × sex combination as the treatment.

To determine whether the population size structure differed between males and females and between PR and STX, we used separate Kolmogorov–Smirnov (K–S) tests to evaluate the following null hypotheses: (1) overall size frequency distributions did not differ between males and females; (2) size frequency distributions did not differ between males and females from PR; and (3) size frequency distributions did not differ between males and females from USVI. Statistical analyses were conducted in SPSS (IBM 2012) and R (RStudio Team 2013). Results were considered significant at P-values less than 0.05. When assumptions for statistical tests were not met, the data were log transformed unless otherwise specified.

Reproduction.— Gonads were removed from each Queen Triggerfish sample; either the whole gonad or the posterior portion of each gonad was fixed in 11% seawater-buffered formalin, Davidson's fixative (Howard et al. 2004), or polyethylene glycol–ethyl alcohol–glycerol–acetic acid (PAGA) fixative (Zanini et al. 2012) for up to 2 weeks and then transferred to 70% isopropanol. Gonad samples were processed using standard histological procedures for

TABLE 1. Summary of Queen Triggerfish sample collections from Puerto Rico (PR) and St. Croix (STX) by fishing method.

Method	Fishery-dependent	Fishery-independent	Total
	PR		
Hook and line	5	37	42
Net	121	–	121
Trap	209	–	209
Spear	193	16	209
Total	528	53	581
	STX		
Trap	60	–	60
Spear	360	147	507
Total	420	147	567

TABLE 2. Histological criteria for Queen Triggerfish gonads during each phase of the reproductive cycle, as modified from Kelly-Stormer et al. (2017). Photographic examples of each phase are provided in Supplementary Figures S-1 and S-2 available in the online version of this article.

Reproductive phase	Male	Female
Immature (never spawned)	Small transverse section compared to regenerating males; little or no spermatocyte development.	Small ovaries. Primary-growth (PG) oocytes only; no evidence of atresia. In comparison with regenerating females, most PG oocytes are less than 60 μm. Area of the transverse section of ovary is smaller; lamellae lack muscle and connective tissue bundles and are not as elongate; germinal epithelium along the margin of lamellae is thicker; and the ovarian wall is thinner. Oogonia are abundant along the margin of lamellae.
Developing	Limited spermatogenesis in testes; elongation of lobules and some development of spermatozoa in testes, but no accumulation in lobules, efferent ducts, and spermatic ducts.	*Early*: Previtellogenic, with only PG and cortical alveolar oocytes. *Middle to late*: Vitellogenic, most advanced oocytes in the yolk granule stage (Vtg1) or yolk globule stage (Vtg2). Oocytes are 170–300 μm in diameter.
Spawning capable	*Early*: Spermatozoa are evident in ducts; spermatogenesis amount in testes ranges from limited to extensive. Greater area of structural tissue in ducts compared to sinuses. *Middle (storage)*: Storage of spermatozoa within expanding ducts; over 50% of the sinuses' area is densely packed with spermatozoa; amount of spermatogenesis in the testes ranges from limited to extensive. *Late (recent spawn)*: Large, expanded ducts are not as densely packed with spermatozoa. Area of sinuses is greater than that of structural tissue. Empty lobules are usually present toward the center of the testes.	Oocyte maturation in the most advanced oocytes: zona radiata becomes thin, and oocytes are undergoing coalescence of yolk globules (Vtg3), germinal vesicle migration (GVM), germinal vesicle breakdown (GVBD), hydration, or ovulation. Postovulatory follicle complexes are sometimes present. Atresia of vitellogenic and/or hydrated oocytes may be present. *Actively spawning subphase*: presence of hydrated oocytes, late GVM, and GVBD.
Regressing	Limited spermatogenesis in testes; some residual spermatozoa in shrunken ducts/lobules and sinuses. Overall number of ducts containing spermatozoa is small. Increase in connective tissue in testes, proliferating from the center.	More than 50% of vitellogenic oocytes with alpha- or beta-stage atresia.
Regenerating	Little or no spermatocyte development; ducts/lobules and sinuses are empty. Large transverse section compared to those of immature males.	Primary-growth oocytes only; traces of atresia. In comparison with immature females, most of the PG oocytes are larger than 60 μm, the area of the transverse section of ovary is larger, lamellae have muscle and connective tissue bundles and are more elongate and convoluted, epithelium along the margin of lamellae is thinner, and the ovarian wall is thicker.
Mature specimen, phase unknown	Mature, but the inadequate; quantity of tissue or postmortem histolysis prevents further assessment of reproductive phase.	Mature, but the inadequate quantity of tissue or postmortem histolysis prevents further assessment of reproductive phase.

triggerfish species (Lang and Fitzhugh 2015; Kelly-Stormer et al. 2017). The tissue samples were vacuum-infiltrated and blocked in paraffin wax. At least three transverse sections (~7 μm thick) were cut using a rotary microtome, mounted on glass slides, stained with double-strength Gill hematoxylin, and counter-stained with eosin-y.

Stained sections were viewed using a compound microscope to determine sex and reproductive phase, assessed according to a modified version of the histological criteria developed for Gray Triggerfish (Table 2; Figures S-1, S-2). Two readers independently assigned sex and reproductive state without knowledge of the capture date, specimen length, or specimen age. If differences in the assignment of reproductive phases occurred, readers examined the slide simultaneously to obtain a consensus phase assignment. If no consensus was reached, then that specimen was eliminated from the analyses. Similar to what was previously observed in Gray Triggerfish (Kelly-Stormer et al. 2017), we noted that the gonads of male Queen Triggerfish were unique in their structure and function compared to those of other reef fish species, and so we documented the male gonad structure and noted its relevance in assigning reproductive phase for males (Table 2; Figures 1, S-2).

To qualitatively determine whether immature and early developing/regenerating specimens were assigned correctly, we compared the size frequency distributions of fish that were definitely mature (developing, spawning capable, or regressing) to the size frequency distributions of immature and early developing/regenerating fish (Harris et al. 2007; Kelly-Stormer et al. 2017). Fish of uncertain sex and reproductive phase were excluded from this comparison. Complete overlap in the left tails of length frequency histograms for definitely mature specimens and early developing/regenerating specimens would provide support for correct assignments of phase for adults without oocytes undergoing vitellogenesis. Minimal overlap between the length histograms for immature and early developing/regenerating specimens would serve as additional support for correct assignment of phases. Specimens with developing, spawning-capable, regressing, and regenerating characteristics were considered sexually mature.

Sex ratios were calculated for PR and STX. Chi-square tests were used to determine whether sex ratios were significantly different from an expected ratio of 1:1. We used generalized linear models fitted to logistic curves to estimate the length at 50% maturity (L_{50}) separately for males and females by island.

The gonadosomatic index (GSI) was determined for sexually mature males and females from each island as follows: GSI = [(gonad weight)/(total weight)] × 100. Mean values for GSI were calculated by month of collection for each sex and each island to examine trends in reproduction and spawning as related to histology. Separate one-factor ANOVAs were used for each island to test the null hypothesis that no significant difference existed in monthly GSI (females and males were tested separately). The GSI values were log transformed to meet the assumptions of normality, and Dunnett's T3 post hoc comparisons were used to examine pairwise significant differences for GSI between months. Additionally, the percentages of individuals that were assigned to each reproductive phase based on the month of collection were plotted separately for males and females by island to visually assess the spawning season duration.

The monthly proportion of spawning-capable females relative to mature females was calculated by island for each month to determine the months of peak spawning. Spawning fraction was calculated for each island by determining the proportion of actively spawning females relative to the total number of mature females. Spawning interval was calculated using the postovulatory follicle (POF)/hydrated-oocyte method (DeMartini and Fountain 1981; Fitzhugh et al. 1993). Females were considered actively spawning if they were undergoing oocyte maturation (germinal vesicle migration through hydration). To calculate spawning interval for females by island, the following equation was used: spawning interval = 1/[(number of actively spawning females)/(number of mature females)].

Oocyte stage and diameter in female Queen Triggerfish were used to determine oocyte development (group-synchronous versus asynchronous) and fecundity type (determinate versus indeterminate). The main criteria used to determine whether a species exhibits group-synchronous development and determinate fecundity follow Hunter et al. (1992) and Greer Walker et al. (1994) and include (1) a hiatus in the size distribution of developing oocytes, (2) a decrease in the number of secondary-growth oocytes through the spawning season, (3) a seasonal increase in secondary-oocyte mean diameter, and (4) atresia that is not generalized at the end of the spawning season, and if present, is distributed sparsely over the season. For oocyte measurements, we randomly selected four females from each island across the spawning season with gonads that were late developing to spawning capable. To obtain a true representation of oocyte count with minimum bias toward a particular oocyte size, counts were made of all oocytes present in 10 microscopic fields using a 4× objective. Oocyte size, obtained by calculating the mean of three diameter measurements for each oocyte, was only recorded for oocytes sectioned through the nucleus. Measurements ranged from 146 to 291 oocytes per gonad (mean = 209). Oocyte size frequency histograms were used to assess a hiatus in oocyte development (criterion 1). To determine whether mean oocyte diameter increased as the spawning season progressed (criterion 2), we used linear regression. For each of the eight ovary samples, we

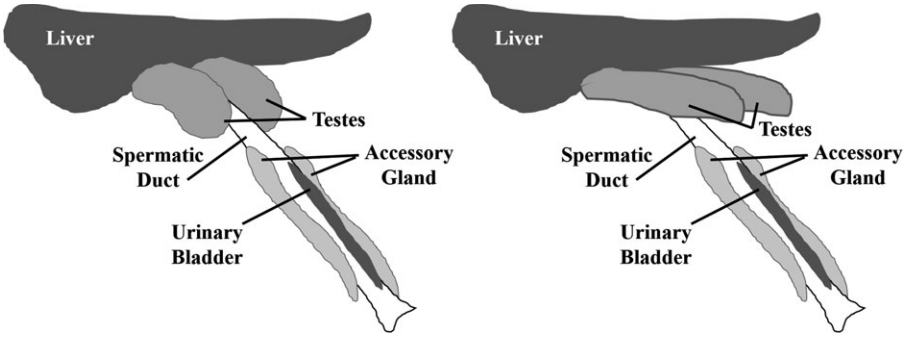

FIGURE 1. Diagrams of male gonads for Gray Triggerfish (left) and Queen Triggerfish (right) relative to some of the other internal organs, illustrating the locations of the testes, spermatic duct, accessory gland, and urinary bladder. Note the general differences in testis shape between the species. The Gray Triggerfish diagram was previously published in Kelly-Stormer et al. (2017); both diagrams were originally made by V. Shervette.

estimated the day of sample collection within the spawning season by calculating the number of days between (1) the sample collection date and (2) the full moon in the December prior to the sample collection date. For example, in 2015, the full moon occurred on December 25, so for a sample collected on July 21, 2016, the number of days between the dates is 209. Spawning season day was then used as the independent variable and the mean diameter of secondary oocytes in each sample was used as the dependent variable for the regression analysis.

RESULTS

Fish Collection

Queen Triggerfish sampling occurred from 2013 to 2018 in waters of PR and STX, USVI. Overall, 1,148 fish were collected: 581 from PR and 567 from STX (Table 3). Mean sizes of females and males from PR were 281 and 299 mm FL, respectively; mean sizes of females and males from STX were 277 and 302 mm FL (Table 3). Overall, males were significantly larger than females; no significant difference in size occurred between PR and STX (Table 4). Linear regression analyses indicated a significant relationship between FL (mm) and weight (g) for females and males sampled from PR and STX (Figure 2; see Table 5 for length–weight equations). The high R^2 values for PR and STX indicated that weight was closely associated with length ($R^2 = 0.97$–0.99; Table 5). This relationship did not differ significantly between sexes or islands (ANCOVA: $F_{3,\ 1,107} = 2.46$, $P = 0.07$).

The overall size frequency distributions were significantly different between males and females (islands combined), with a larger proportion of males in the larger size-classes (K–S test: $Z = 3.84$, $P < 0.001$; Figure 3; Table 6). The size frequency distributions of males and females were also significantly different in PR ($Z = 1.77$, $P = 0.004$) and in STX ($Z = 3.97$, $P < 0.001$).

Reproduction

In total, 1,138 gonads from Queen Triggerfish were collected. Sex and reproductive phase were assigned to 1,120 (98%) individuals. In general, for many fish species, the male and female gonads have relatively similar anatomy in that they consist of two posteriorly attached lobes and release gametes (through the oviduct for females and the spermatic duct for males; Parenti and Grier 2004). Gonads of female triggerfish are similar in shape to female gonads from other fish species (Kelly-Stormer et al. 2017). For male Queen Triggerfish, we documented that the gonad anatomy and structure were similar to those of Gray Triggerfish (Kelly-Stormer et al. 2017); the male gonads consist of testes, spermatic duct, and accessory glands (Figures 1, S-2). The accessory glands are important because they store spermatozoa before spawning. One minor difference between male Queen Triggerfish and male Gray Triggerfish was that the testes were more elongate in Queen Triggerfish compared to the more kidney-bean-shaped testes in Gray Triggerfish (Figure 1). We found that in order to assign the most accurate reproductive state to male Queen Triggerfish, a close examination of the testes and accessory glands was necessary (Table 2).

Immature Queen Triggerfish comprised 16% of the total specimens for which reproductive phase was determined. Correct assignment of reproductive tissue to the immature and early developing/regenerating gonad categories was indicated by (1) the complete or near-complete overlap in the left tail of length histograms for definitely mature (i.e., developing, spawning capable, and regressing) and early developing/regenerating specimens and (2) the minimal overlap in histograms for immature and early developing/regenerating specimens (Figure 4).

The overall female : male sex ratio for samples from PR was 1.0:1.3, which differed from the expected 1:1 ratio ($\chi^2 = 11.4$, df = 1, $P = 0.001$). In STX samples, the sex ratio was not significantly different from 1:1 ($\chi^2 = 0.2$, df = 1, $P = 0.672$).

For PR samples, the smallest mature male was 196 mm FL, and the largest immature male was 230 mm FL (Table 7). The L_{50} of PR males was 206 mm FL (Table 7; Figure 5). The smallest mature female sampled from PR was 215 mm FL, and the largest immature female was 285 mm FL. The L_{50} of PR females was 256 mm FL (95% confidence interval = 246–264 mm), and all females larger than 276–300 mm FL were sexually mature (Table 7; Figure 5). For STX samples, the smallest mature male was 184 mm FL, and the largest immature male was 253 mm FL (Table 7). The L_{50} of STX males was 211 mm FL, and all males larger than 251–275 mm FL were mature (Table 7; Figure 5). The smallest mature STX female was 219 mm FL, and the largest immature STX female was 329 mm FL. The L_{50} of STX females was 245 mm FL, and all females were mature by 351–375 mm FL (Figure 5).

Monthly GSI was calculated separately for females and males from PR and STX (Figure 6). A significant difference in the mean female GSI value among months occurred for PR (ANOVA: $P < 0.001$) and STX ($P < 0.001$; Table 8). Pairwise post hoc comparisons revealed that for females in PR, mean GSI was significantly higher in December compared to February, May, July, September, and October (Dunnett's T3: $P < 0.03$; Figure 6). In STX, female mean GSI was significantly higher in January compared to June, September, October, and November; February compared to March–June and September–December; and March compared to September–November ($P < 0.02$; Figure 6). Mean monthly GSI significantly differed among months for PR males ($P < 0.001$) and for STX males ($P < 0.001$; Table 8). Post hoc comparisons revealed that for males in PR, mean GSI was significantly higher in February compared to July–November; March compared to September–October; and June compared to July–November ($P < 0.02$; Figure 6). In STX, male mean GSI was significantly higher in January compared to March; February compared to July and November; March compared to May–September and November–December; April compared to July and November; and May compared to July–September and November ($P < 0.04$; Figure 6). The monthly percentage of spawning-capable Queen Triggerfish samples peaked during December and January in PR and during January and February in STX (Table 9).

Based on the PR data set for the period 2013–2018, the beginning of the spawning season was December 24, which was the earliest date that oocyte maturation was observed in females during any year. The end of the spawning season in PR was August 22, which was the

TABLE 3. Overview of capture depth (m) and size (FL, mm) for male and female Queen Triggerfish, including the total number of fish sampled from Puerto Rico (PR) and St. Croix (STX) and the percentages of males, females, and unknown sex.

Variable	PR	STX
Depth range (m)	2–90	2–30
Total number of fish	581	567
Percent male	55	50
Percent female	41	48
Percent unknown	4	2
Size range (FL; mean)	67–434 (291)	190–414 (291)
Male FL range (mean)	67–433 (299)	191–402 (302)
Female FL range (mean)	109–434 (281)	190–414 (277)

TABLE 4. Results from ANOVA testing for significant differences in mean size (FL, mm) of Queen Triggerfish.

Source	df	Mean square	F	P
Sex	1	127,801	51.042	<0.001
Island	1	2	0.001	0.980
Island × sex	1	4,790	1.913	0.167
Error	1,116	2,504		

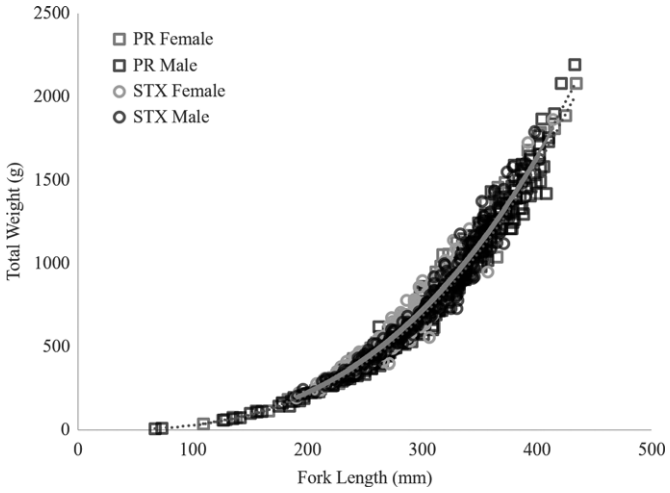

FIGURE 2. Queen Triggerfish FL versus weight for females and males from Puerto Rico (PR) and St. Croix (STX).

TABLE 5. Linear regression equations describing length–weight relationships for female and male Queen Triggerfish from Puerto Rico (PR) and St. Croix (STX).

Island and sex	Equation	R^2
PR female	$y = (5 \times 10^{-5})x^{2.9018}$	0.99
PR male	$y = (4 \times 10^{-5})x^{2.9231}$	0.99
STX female	$y = (7 \times 10^{-5})x^{2.8238}$	0.99
STX male	$y = (5 \times 10^{-5})x^{2.8892}$	0.97

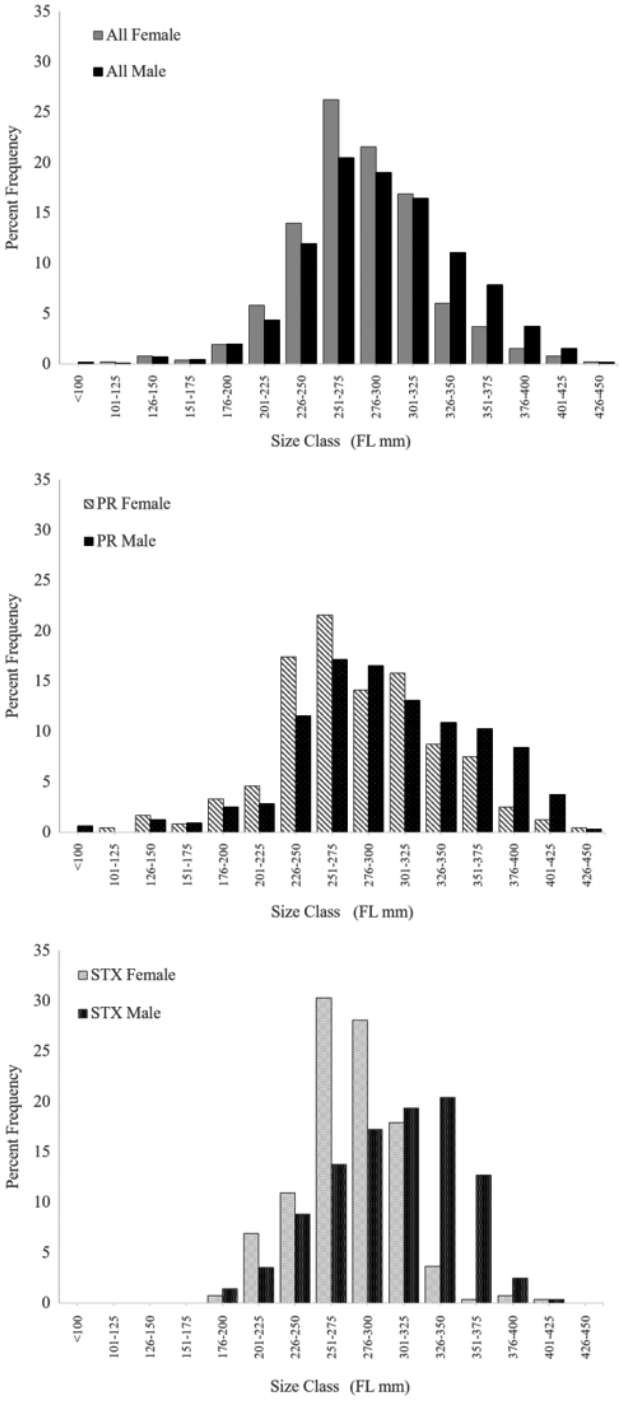

FIGURE 3. Size-class distributions for Queen Triggerfish females and males from Puerto Rico (PR) and St. Croix (STX) combined and from each island separately.

latest date that late-developing oocytes and POFs occurred in females observed in any year. This results in a PR spawning season of 241 d (Figure 7). Based on the STX data set from 2015 to 2018, the earliest date that oocyte maturation was observed in females in any month was December 11, and the end of the spawning season (the latest date on which late-developing oocytes were observed) was August 9. This yields an STX spawning season of 241 d (Figure 7). However, STX fishers reported observing nest guarding by female Queen Triggerfish as early as the week after the full moon in December (Shervette, unpublished data) and as late as the week after the full moon in August. If Queen Triggerfish time their spawning events around the full moon starting in December and continue to spawn through the full moon in August, then that yields a maximum spawning season of 267 d. Spawning-capable males occurred at relatively high proportions during every month in both PR and STX (Figure 8).

Only three females in PR and three females in STX had gonads with hydrated oocytes or early POFs. This yielded a rounded spawning fraction value of 0.02 for both PR (3/164) and STX (3/169; Table 10). Spawning interval for PR females was approximately every 54 d (or 1/0.02, the reciprocal of the overall proportion of spawning females, expressed in days); for STX females, the spawning interval was approximately 56 d. With a spawning season ranging from 241 to 267 d in PR and STX, a

TABLE 6. Results of Kolmogorov–Smirnov tests for differences in size frequency distributions between male and female Queen Triggerfish sampled in Puerto Rico (PR) and St. Croix (STX).

Comparison	*Z*-statistic	*P*
Overall females versus males	3.84	<0.001
PR females versus males	1.77	0.004
STX females versus males	3.97	<0.001

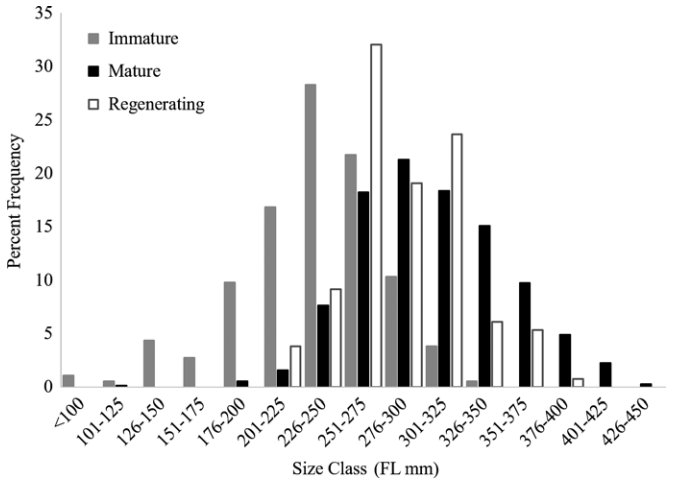

FIGURE 4. Size frequency distributions of Queen Triggerfish females and males with gonads categorized as immature, mature (developing, spawning capable, or regressing), or regenerating.

female Queen Triggerfish can potentially spawn four to five times in the season (Table 10).

The individual oocyte size frequencies of the eight randomly selected females (four from PR and four from STX) exhibited bimodal distributions (Figure S-3), all showing a clear hiatus between the sizes of oocytes in the cortical alveolar stage and the sizes of those in the vitellogenic stage (Figure S-3). This is indicative of a group-synchronous pattern of oocyte development and is normally indicative of determinate fecundity. However, mean secondary-oocyte diameter did not increase significantly with spawning season day (linear regression: $r^2 = 0.02$, $P = 0.142$); this finding means that the Queen Triggerfish does not meet the criteria for determinate fecundity.

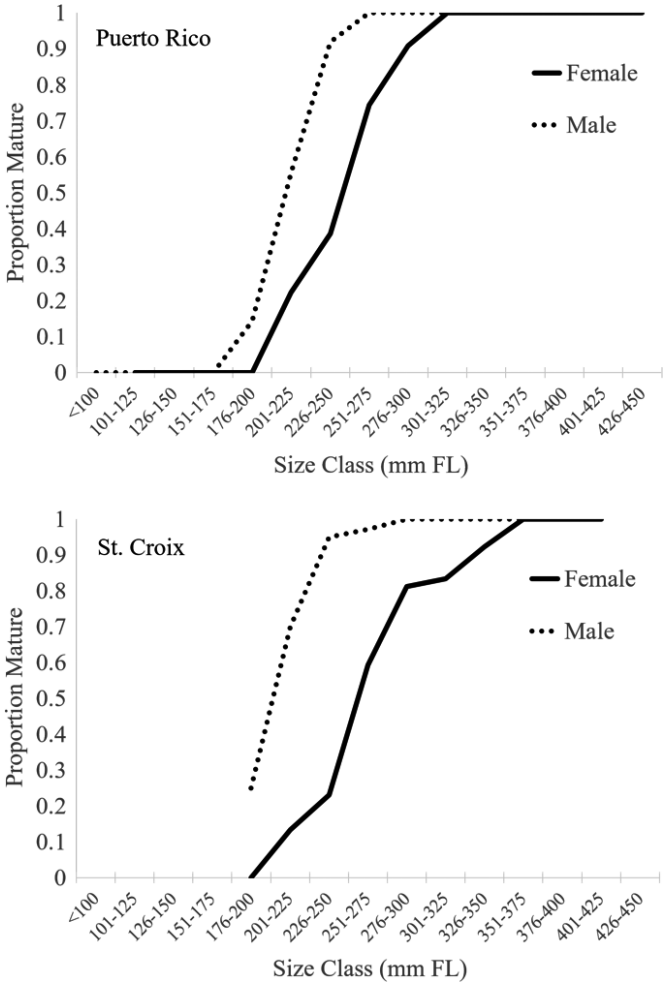

FIGURE 5. Female and male 50% sexual maturity curves for Queen Triggerfish sampled from Puerto Rico and St. Croix.

DISCUSSION

Our study provides important life history information for an exploited population of Queen Triggerfish. It is the first to describe comprehensively the reproductive biology of this species by using histological methods. Similar to other species in the Balistidae family, Queen Triggerfish in the U.S. Caribbean spawn in pairs, establish and defend nesting territories, and protect and care for their fertilized eggs (Fricke 1980; Ishihara and Kuwamura 1996; Kuwamura 1997; Kawase 2003; Simmons and Szedlmayer 2012). The mean length of Queen Triggerfish males was significantly larger than that of females in PR and STX. Similar findings have been reported for Gray Triggerfish populations, in which males are significantly larger than females, attaining a larger size at age and a greater asymptotic length (Hood and Johnson 1997; Ingram 2001; Kelly-Stormer et al. 2017). To some degree, these differences in size between males and females in triggerfish species may relate to their mating and nesting strategies (Fricke 1980; Gladstone 1994; Kawase 2003; Simmons and Szedlmayer 2012). Kawase (2003) documented that male Redtail Triggerfish *Xanthichthys mento* established and defended territories before spawning and during egg care around the Izu Islands of Japan. Females nesting in a male's territory focused only on caring for the fertilized eggs and guarding them (Kawase 2003). Similar reproductive behavior has been reported for Gray Triggerfish in the northern Gulf of Mexico, where a large, dominant male patrols a nesting territory, builds and maintains several nests, and guards the nesting area after fertilization (Simmons and Szedlmayer 2012). The larger size of males may prove advantageous in defending the territory and nests, thus optimizing the potential survival of the developing embryos and the fertilizing male's contribution to the next generation.

The Queen Triggerfish is a gonochoristic species, and females exhibit group-synchronous oocyte development, which is usually associated with determinate fecundity (McBride et al. 2015). In contrast, we found evidence that Queen Triggerfish have indeterminate fecundity, because the mean diameter of vitellogenic oocytes did not increase as the spawning season progressed. Lang and Fitzhugh

TABLE 7. Sizes at sexual maturity for Queen Triggerfish males and females from Puerto Rico (PR) and St. Croix (STX). Maximum (max) size of immature fish, minimum (min) size of mature fish, and length at 50% maturity (L_{50}; with 95% confidence interval [CI]) are shown.

Island and sex	Immature max size (mm FL)	Mature min size (mm FL)	L_{50} (95% CI)
PR female	285	215	256 (246–264)
PR male	230	196	206 (180–218)
STX female	329	219	245 (238–251)
STX male	253	184	211 (196–220)

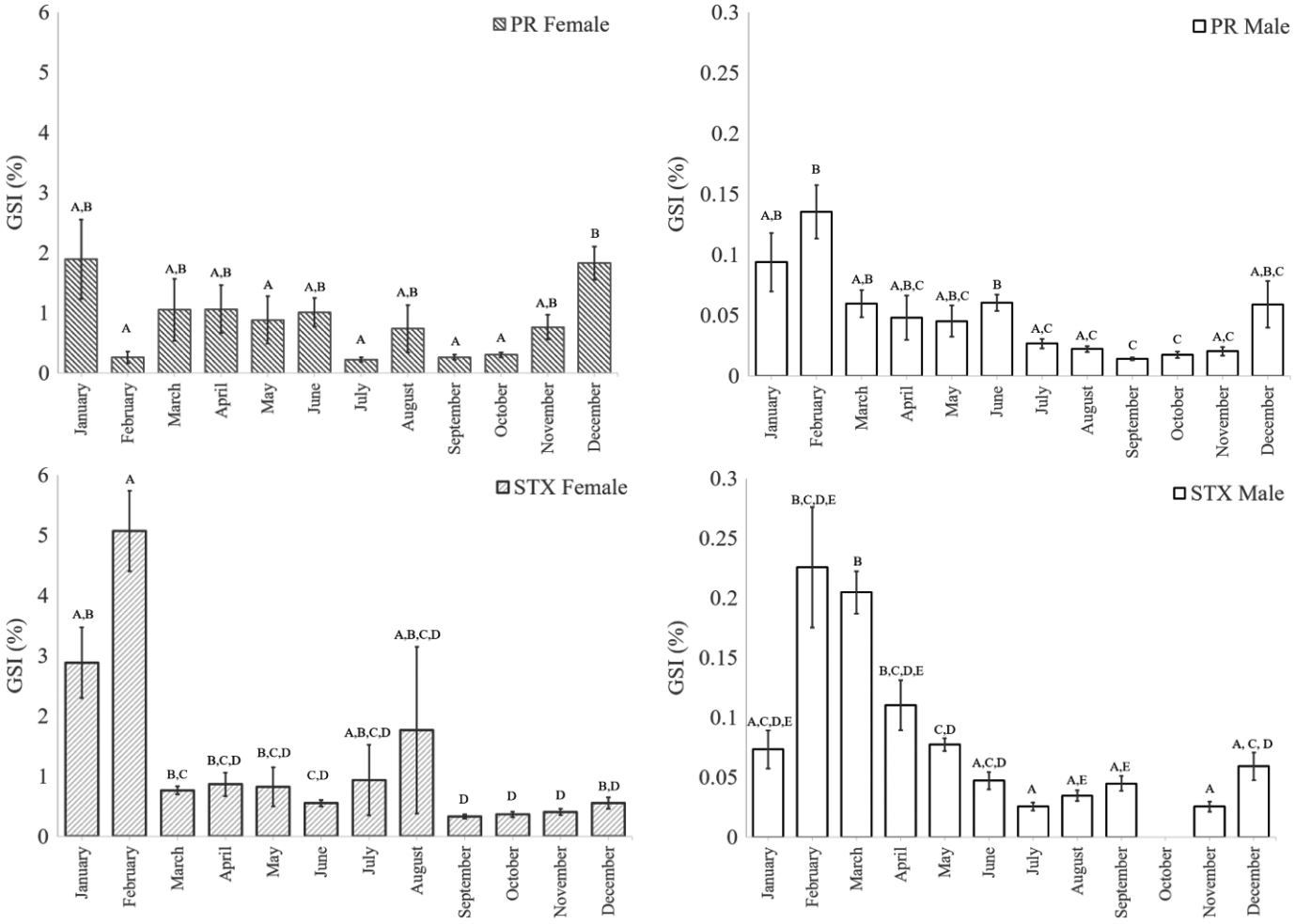

FIGURE 6. Mean (±SE) monthly gonadosomatic index (GSI) values for sexually mature Queen Triggerfish females and males from Puerto Rico (PR) and St. Croix (STX). Letters within each graph indicate significant differences in monthly mean GSI for pairwise comparisons ($\alpha = 0.05$). No mean value is presented for STX males in October because the scale for weighing gonads (nearest 0.01 g) malfunctioned before testes could be weighed.

TABLE 8. Analysis of variance results for gonadosomatic index values of Queen Triggerfish by month in Puerto Rico (PR) and St. Croix (STX).

Island and sex	Source	df	Mean square	*F*	*P*
PR female	Month	11	0.14	3.82	<0.001
	Error	155	0.04		
PR male	Month	11	0.03	7.35	<0.001
	Error	237	0.01		
STX female	Month	11	0.39	10.28	<0.001
	Error	164	0.04		
STX male	Month	10	0.09	15.64	<0.001
	Error	216	0.01		

(2015) reported similarly contradictory evidence concerning fecundity type for female Gray Triggerfish and ultimately concluded that Gray Triggerfish have indeterminate fecundity. Although uncommon, group-synchronous oocyte development combined with indeterminate fecundity has been documented in a few other fish species (Nakazono 1993; Yoneda et al. 1998; Ganias et al. 2004), including the Spiny Chromis *Acanthochromis polyacanthus*, another demersal egg-laying, brood-caring reef fish (Nakazono 1993). Male and female Spiny Chromis exhibit monogamy and bi-parental care of broods. Nakazono (1993) conducted parent removal experiments with this damselfish species and reported that fry survival diminished significantly when one parent was removed. Females who lost their male partners would ultimately abandon the brood and take a new partner. Because Spiny Chromis females have indeterminate fecundity, they were able to produce a second brood with their new partner (Nakazono 1993). Compared to the majority of fisheries-targeted reef fish species, Queen Triggerfish and Gray

TABLE 9. Percentage of female Queen Triggerfish in the spawning-capable phase ([number of spawners]/[number of mature females sampled]) for each month they occurred in Puerto Rico (PR) and St. Croix (STX).

Month	PR	STX
Dec	33% (3/9)	5% (1/19)
Jan	21% (5/24)	35% (10/29)
Feb	–	73% (8/11)
Mar	11% (2/18)	5% (1/19)
Apr	7% (1/14)	6% (2/34)
May	7% (1/15)	8% (1/13)
Jun	21% (4/19)	–
Jul	–	6% (1/15)
Aug	4% (1/23)	14% (1/7)

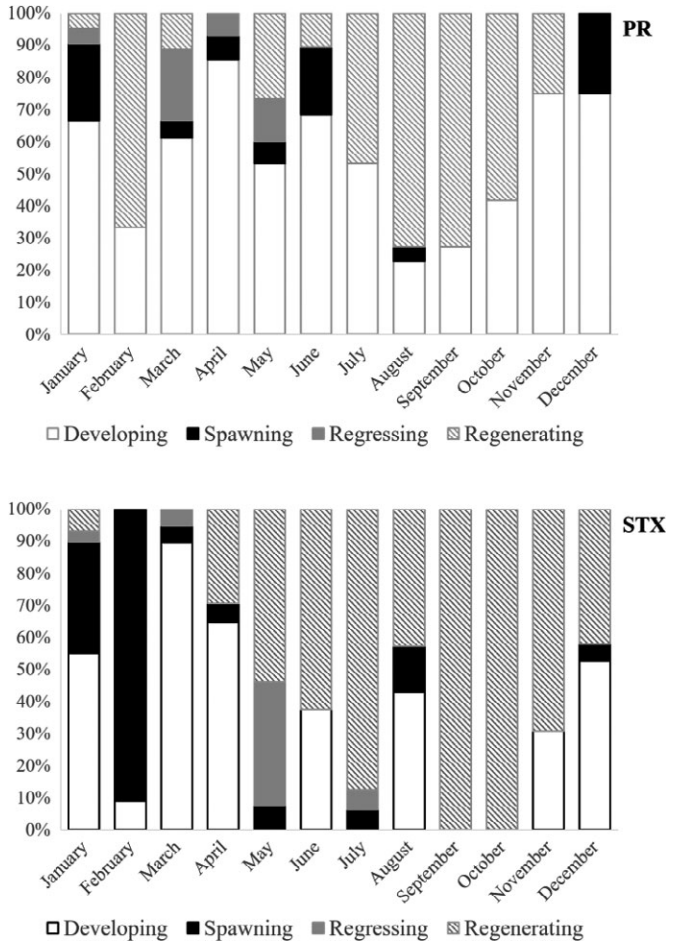

FIGURE 7. Reproductive seasonality of female Queen Triggerfish in Puerto Rico (PR) and St. Croix (STX). Monthly proportions of individual females in each reproductive phase are presented.

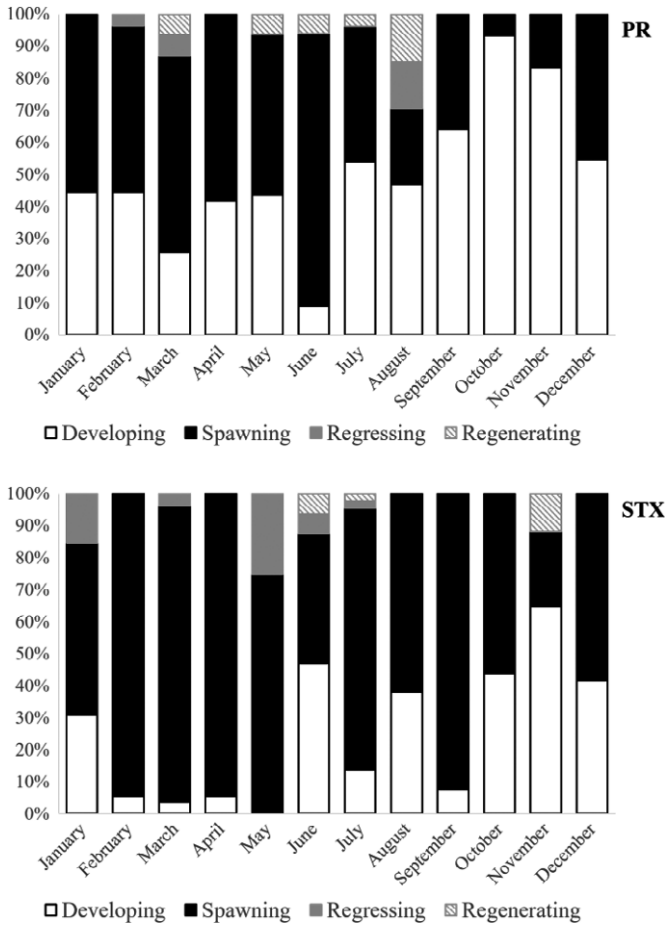

FIGURE 8. Reproductive seasonality of male Queen Triggerfish in Puerto Rico (PR) and St. Croix (STX). Monthly proportions of individual males in each reproductive phase are presented.

TABLE 10. Spawning fraction, spawning interval, and spawning frequency for female Queen Triggerfish from Puerto Rico (PR) and St. Croix (STX). The number of calendar days from the full moon in December to the full moon in August is 267. In PR, the first spawning-capable female was sampled on December 24, and the last spawning-capable female was observed on August 22, yielding a total of 241 d. In STX, the first spawning-capable female was observed on December 11, and the last spawning-capable female was sampled on August 9, yielding a total of 241 d.

Metric	PR	STX
Spawning fraction	0.02 (3/164)	0.02 (3/169)
Spawning interval (d)	54	55
Spawning frequency (number of times/year)	4–5	4–5

Triggerfish are atypical in their demersal nesting and brood care. The combination of group-synchronous oocyte development and indeterminate fecundity may be rare in the majority of reef fisheries species that are pelagic spawners but may be more common in demersal egg-laying species that care for their broods.

We found that Queen Triggerfish in U.S. Caribbean waters spawned from December to August based on the occurrence of spawning-capable females during those months and that their spawning activities seemed to be associated with the full moon. Spawning activities associated with a specific lunar phase have been documented in other Caribbean reef fish species, including the Red Hind *Epinephelus guttatus* (Sadovy et al. 1994), Nassau Grouper *E. striatus* (Smith 1972), Dog Snapper *Lutjanus jocu*, and Cubera Snapper *L. cyanopterus* (Biggs and Nemeth 2016). We did not design our monthly fish sampling in relation to the lunar cycle, so starting in December 2017, we asked STX spear-fishers about their underwater observations on Queen Triggerfish behavior. The spear-fishers reported observing Queen Triggerfish guarding shallow nests for up to 1 week after the full moon in December 2017 and in January, February, and March 2018. Lunar-cycle-driven spawning activities have been observed in other triggerfish species (Gladstone 1994; Donaldson and Dimalanta 2011). Future Queen Triggerfish sampling efforts for both islands are needed to further investigate and confirm spawning activities as they relate to the lunar monthly cycle.

Only one main study has examined the reproductive biology of Queen Triggerfish in the Caribbean. Aiken (1983) obtained fishery-independent Queen Triggerfish samples in 1969–1973 from the waters of Jamaica. Combining the macroscopic observations of gonads from males and females, Aiken (1983) reported that the reproductive season for Queen Triggerfish was January–March, May, and July–December. Our study does not support those initial findings (Figure 8). Although a proportion of the Queen Triggerfish males were spawning capable each month of the year in our study, the actual spawning season is determined by the occurrence of spawning-capable females in the population (Murua and Saborido-Rey 2003). In PR and STX combined, spawning-capable females occurred as early as December and as late as August. In PR, we had difficulty in obtaining medium-sized Queen Triggerfish samples during February of each year due to recurring current and wind patterns that prevented fishers from fishing; over the period 2013–2018, we only managed to collect four mature females in February (out of a total of 35 samples from PR February collections). However, spawning-capable females did occur at a high proportion in February collections from STX.

The spawning season for Queen Triggerfish lasts longer than the spawning seasons of Gray Triggerfish populations from around the Atlantic Ocean (Ofori-Danson 1990; Bernardes and Dias 2000; Kacem and Neifar 2014; Lang and Fitzhugh 2015; Kelly-Stormer et al. 2017). Length of the spawning season is an important determinant of reproductive success (Anderson et al. 2008; Wright and Trippel 2009). A combination of several factors may explain the differences in timing and length of spawning seasons among studies. Although they are congeners, Queen Triggerfish and Gray Triggerfish are two different species. Furthermore, differences in the sampling design of the studies and the methods used to estimate reproductive seasonality can result in different findings (Lowerre-Barbieri et al. 2011). Overall, regional variation in temperature, community composition, habitat complexity, and fishing pressure may play a role in regulating the reproductive seasonality of fish populations.

Spawning frequency estimation is critical for quantifying fecundity in species with indeterminate fecundity (Hunter et al. 1992; Murua and Saborido-Rey 2003; Ganias 2009). We estimated that female Queen Triggerfish could spawn four to five times throughout the spawning season (Table 10), which is half the spawning frequency estimated for Gray Triggerfish (Lang and Fitzhugh 2015; Kelly-Stormer et al. 2017). Queen Triggerfish and Gray Triggerfish share a relatively unique reproductive strategy compared to other medium- to large-bodied, fisheries-targeted species (Johannes 1978; Lambert and Ware 1984). The combined benefits of parental investments in maintaining territories, benthic nesting and guarding (Simmons and Szedlmayer 2012), a protracted spawning season with multiple spawning events, and relatively high fecundity that increases with size (Lang and Fitzhugh 2015) may result in higher survival rates for larval triggerfish in comparison with the larvae of pelagic spawners. Additional research on Queen Triggerfish larval habitat use and survival is necessary to verify this.

In summary, the current study provides critical information on Queen Triggerfish populations in the U.S. Caribbean and provides fisheries managers with a comprehensive understanding of the spawning season, fecundity type, and size at maturity for males and females. Future research on Queen Triggerfish should focus on determining the population age structure and age at maturity, discovering the locations of spawning grounds that could be seasonally protected, and the relationship between monthly spawning patterns and the lunar cycle. Fisheries managers should examine the potential impacts of fishing pressure on Queen Triggerfish in the U.S. Caribbean and incorporate any region-specific differences in reproductive season, size and age at maturity, and population demographics into the establishment and enforcement of management regulations for this species.

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Explanation of authors' contributions: this paper is based on the Master of Science thesis of J.M.R.H. As the principal investigator of the larger study on reef fish life history and as the research advisor, V.S. worked closely with J.M.R.H. in

the organization and writing of the thesis and co-wrote this paper. N.P.A. taught and mentored J.M.R.H. on fish reproduction and histology and provided guidance and assistance in the collection and analyses of PR reproduction data. K.C.V. assisted in sample collection, processing, database management, and manuscript review. R.A. was J.M.R.H.'s thesis advisor; R.A. and R.N. were co-investigators on the larger reef life history study with V.S. and provided important direction, resources, and feedback during the development of the project. This study was funded by a National Oceanic and Atmospheric Administration Marine Fisheries Initiative Grant awarded to V.S., N.P.A., R.A., and R.N. (NA11NMF4330130; National Marine Fisheries Service); the Department of Biology and Geology at the University of South Carolina (USC), Aiken; and the USC Office of Research. We thank Amanda Kelly-Stormer for initial guidance in reading triggerfish histology slides. This work would not have been possible without the extensive assistance of the Fisheries Research Laboratory at the Puerto Rico Departamento de Recursos Naturales y Ambientales (Wilfredo Torres, Grisel Rodríguez, Verónica Seda, Luis A. Rivera, and Wilson Santiago), The Nature Conservancy in STX, the fishers that collaborated with us to obtain samples (Gerardo Ramirez, Pedro Silva, Benigno Rodríguez, Felix Diaz, Gerson Martínez, Bobby Thomas, and Felix Lugo), and the people that assisted during processing in PR and STX (Kayley Kirkland, Kristin Garlick, Sara Thomas, Graham Wagner, and Jeff Lokken). There is no conflict of interest declared in this article.

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.