Queen Triggerfish *Balistes vetula* Reproductive Biology in US Caribbean Waters

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By

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ABSTRACT

Effective fisheries management requires a detailed understanding of the life history strategies of managed species. Queen Triggerfish *Balistes vetula* supports productive fisheries in the western Atlantic, including in the U.S. Caribbean. We utilized a combination of fisheries-dependent and –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 2013-2018, 1148 samples were collected ranging in size from 67-434 mm fork length (FL). Results from this study provide important life history information for an exploited population and this study is the first to describe Queen Triggerfish reproductive biology in detail for the Caribbean. We documented that 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. Puerto Rico and St. Croix, USVI, male 50% size-at-sexual maturity (L_{50}) were similar (206 and 211 mm FL, respectively) and were significantly smaller compared to female L_{50} (Puerto Rico: 256 mm FL; St. Croix: 245 mm FL). We also documented that Queen Triggerfish, a nesting benthic spawner, exhibits group-synchronous oogenesis and indeterminate fecundity over the spawning season that starts as early as the week after the full moon in December and extends until August. Spawning interval, defined as the number of days between spawning events in a female, ranged from 54-55 days indicating that a female could spawn up to five times over the estimated 241-267 days spawning season. As regulations in the Caribbean on grouper and snapper species increase, Queen Triggerfish will experience increasing fishing pressure. Managers should continue to evaluate potential impacts of this pressure and establish management regulations that take into consideration the region-specific reproductive season and size-at-maturity.

RESUMEN

La gestión efectiva de la pesca requiere una comprensión detallada de las estrategias de historia de vida de las especies gestionadas. El peje puerco reina *Balistes vetul*a apoya las pesquerías productivas en el Atlántico occidental, incluido el Caribe de los Estados Unidos. Utilizamos una combinación de muestras dependientes de la pesquería e independientes para evaluar la estructura de tallas, la proporción de sexos, el tamaño de madurez, la temporada de desove y la frecuencia de desove de una población de peje puerco reina en el Caribe de EE. UU. De 2013 a 2018, se recogieron 1148 muestras que variaban en tamaño desde 67-434 mm de longitud de horquilla (FL). Los resultados de este estudio proporcionan información importante sobre el ciclo de vida de una población explotada, y este estudio es el primero en describir la biología reproductiva del peje puerco reina en detalle para el Caribe. Documentamos que el peje puerco reina es una especie sexualmente dimórfica caracterizada por un tamaño mediano en la madurez. El macho y la hembra sexualmente maduros más pequeños tenían FL de 184 y 215 mm. Puerto Rico y St. Croix, Islas Vírgenes de los Estados Unidos, el 50% de madurez sexual (L_{50}) fueron similares (206 y 211 mm FL, respectivamente) y fueron significativamente menores en comparación con L_{50} de las hembras (Puerto Rico: 256 mm FL; Croix: 245 mm FL). También documentamos que el peje puerco reina es un engendrador bentónico de anidación, exhibe una ovogénesis sincrónica de grupo y fecundidad indeterminada durante la temporada de desove que comienza a partir de la semana posterior a la luna llena en diciembre y se prolonga hasta agosto. El intervalo de desove, definido como el número de días entre los eventos de desove en una hembra, varió de 54-55 días, lo que indica que una hembra podría reproducirse hasta cinco veces durante la temporada de desove estimada de 241-267 días. A medida que aumenten las regulaciones en el Caribe sobre las especies de mero y pargo, el peje puerco reina experimentará

una creciente presión de pesca. Los oficiales de manejo deben continuar evaluando los impactos potenciales de esta presión y establecer regulaciones de manejo que tomen en consideración la temporada reproductiva específica de la región y el tamaño de madurez.

DEDICATION

To my committee members for their advice:

Dr. Richard S. Appeldoorn

Dr. Virginia R. Shervette

Dr. Jorge R. García-Sais

And for the persons that made the impossible possible, helping me to get the job done when I was unable to be in many places at once:

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INTRODUCTION

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 from the Balistidae family occur in temperate and tropical waters in the Atlantic, Pacific, and Indian Oceans (Matsuura 2015). Species from the genus *Balistes* support important fisheries in the Atlantic Ocean (*B. capriscus* and *B. vetula*), north and south of the equator (Aggrey-Fynn and Sackey-Mensah 2012; Barroso-Soto et al. 2007; Bernardes 2002; NMFS 2009), in the Mediterranean Sea (Kacem et al. 2015; Kacem and Neifar 2014), Gulf of Mexico (SEDAR 2006) and Caribbean Sea (Matos-Caraballo 2012; SEDAR 2013) and in the Pacific Ocean (*B. polylepis* – eastern Pacific). However, little published information exists on the reproductive biology of several *Balistes* species.

Queen Triggerfish *Balistes vetula* is a moderately long-lived species (Albuquerque et al. 2011) and a 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 following small spine is depressed, or "triggered". Queen Triggerfish are distributed in the western Atlantic from as north as North Carolina to as south as Brazil. The distribution and habitat preferences of Queen Triggerfish in many ways is similar to its congener, Gray Triggerfish (*B. capriscus*), 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 fed on sea urchins during daylight hours at an

artificial reef site in the U.S. Virgin Islands (USVI), with the reported Queen Triggerfish size range of 215-330 mm fork length (FL).

Manooch and Drennon (1987) examined age and growth of Queen Triggerfish from Puerto Rico and the U.S. Virgin Islands. They used dorsal spines for aging specimens collected from 1983-1984 and reported that annulus formation occurred from February-March and the maximum age was seven years. Ferreira de 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 (Gladstone 1994; Kawase 2003; Kuwamura 1997; Lobel and Johannes 1980; Simmons and Szedlmayer 2012). Many Balistidae species are characterized by lek-like spawning systems in which a male establishes and defends a nesting territory, males and females construct benthic nests for individual females to lay her eggs in, and adults guard nests and care for the developing embryos after fertilization until larvae emerge. Simmons and Szedlmayer (2012) investigated this reproductive strategy in the closely related congener Gray Triggerfish and documented that an individual female stays inside the nest and guards the eggs while the male guards the territory surrounding the nests. These behaviors continue until the larvae emerge, which occurs within 24-48 hours after fertilization (Simmons and Szedlmayer 2012).

Little information exists concerning the reproductive biology and ecology of Queen Triggerfish in U.S. waters. Ferreira de Menezes (1979) reported that Queen Triggerfish spawn in Brazilian waters mainly in March and April and the size range of sexually mature individuals

was 238-502 mm fork length. Anecdotal information from spear fishers in St. Croix, USVI, indicates that Queen Triggerfish establish and guard nests starting as early as December during the week after the full moon (V. Shervette, unpublished data; G. Martinez, STX spearfisher, personal communication).

In many ways, Puerto Rico 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 portions (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 fisheries species in U.S. Caribbean waters In USVI, it ranks third in reconstructed annual landings from 1950-2010 for commercial reef fish (Ramdeen et al. 2015) and in Puerto Rico it has consistently remained one of the top seven targeted reef fish species over the past 20 years, by pounds landed (Matos-Caraballo 2012; Matos-Caraballo et al. 2007). Annual commercial landings for the region for 2000-2011, summarized by McCarthy (2012), were obtained from fisher logbook reports. In Puerto Rico, Queen Triggerfish landings have been variable since 2000, but generally trended around 60,000-70,000 lbs. (McCarthy 2012). During the same period in USVI (St. Thomas, St. John, and St. Croix), annual commercial landings for "Triggerfishes" increased from 94,905 to a maximum of 131,449 lbs in 2002 and then declined to 55,841 lbs 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, 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 Puerto Rico and USVI waters: 1. size structure and sex ratios; 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 Puerto Rico (PR) and the coastal areas surrounding St. Croix, USVI (STX). We collected Queen Triggerfish samples from a combination of fisheries-dependent and –independent sources (Table 1). Monthly fisheries-dependent samples were purchased from PR and STX fishers between July 2013 and March 2018. For 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. Fisheries-independent samples were collected opportunistically by hook-and-line and spear-fishing. For each fish sample, we measured standard length (SL), fork length (FL), and total length (TL) to the nearest mm and total weight to the nearest g. Gonads were removed and 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 if 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 length (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 if 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.

Table 1. Summary of Queen Triggerfish sample collections by fishing method and source.

Island	Fisheries-Dependent	Fisheries-Independent	Total
Puerto Rico	**528**	**53**	**581**
Hook-and-Line	5	37	42
Net	121	-	121
Trap	209	-	209
Spear	193	16	209
St. Croix	**420**	**147**	**567**
Trap	60	-	60
Spear	360	147	507

Table 2. Histological criteria for Queen Triggerfish gonads during each phase of the reproductive cycle, modified from Kelly-Stormer et al. 2017. Photographic examples of each phase are provided in Figures 1-2.

Reproductive Phase	Male	Female
Immature (never spawned)	Small transverse section compared to regenerating male; little or no spermatocyte development.	Small ovaries. Primary growth oocytes only; no evidence of atresia. In comparison with regenerating female, most primary growth oocytes <60 um. Area of transverse section of ovary is smaller, lamellae lack muscle and connective tissue bundles and are not as elongate, germinal epithelium along margin of lamellae is thicker, ovarian wall is thinner. Oogonia are abundant along 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 primary growth and cortical alveolar oocytes (CA). Middle-Late: Vitellogenic, most advanced oocytes in yolk-granule (Vtg1) or yolk-globule stage (Vtg2). Oocytes 170-400 um in diameter.
Spawning capable	Early: Spermatozoa evident in ducts; spermatogenesis amount in testes ranges from limited to extensive. Greater area of structural tissue in ducts compared to sinuses. Middle (Storage): Spermatozoa storage within expanding ducts; >50% of sinuses' area densely packed with spermatozoa; amount of spermatogenesis in testes ranges from limited to extensive. Late (Recent Spawn): large expanded ducts not as densely packed with spermatozoa. Area of sinuses greater than structural tissue. Empty lobules usually present towards center of 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 sometimes present. Atresia of vitellogenic and/or hydrated oocytes may be present. Actively Spawning Subphase: Presence of hydrated oocytes (HO), late GVM, GVBD
Regressing	Limited spermatogenesis in testes; some residual spermatozoa in shrunken ducts/lobules and sinuses. Overall number of ducts containing spermatozoa small. Increase in connective tissue in testes, proliferating from center.	More than 50% of vitellogenic oocytes with alpha- or beta-stage atresia.
Regenerating	Little or no spermatocyte development; empty ducts/lobules and sinuses. Large transverse section compared to immature male.	Primary growth oocytes only; traces of atresia. In comparison with immature female, most primary growth oocytes >60 um, area of transverse section of ovary is larger, lamellae have muscle and connective tissue bundles, lamellae are more elongate and convoluted, epithelium along margin of lamellae is thinner, ovarian wall is thicker.
Mature specimen, phase unknown	Mature, but inadequate quantity of tissue or postmortem histolysis prevent further assessment of reproductive phase.	Mature, but inadequate quantity of tissue or postmortem histolysis prevent further assessment of reproductive phase.

To determine whether the population size structure differed between males and females and between the Puerto Rico and U.S. Virgin Islands (St. Croix [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 Puerto Rico; 3) size frequency distributions did not differ between males and females from USVI. Statistical analyses were conducted in SPSS. Results were considered significant at p-values < 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 and 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 PAGA fixative (Zanini et al. 2012) for up to two weeks, then transferred to 70% isopropanol. Gonad samples were processed using standard histological procedures for triggerfish species (Kelly-Stormer et al. 2017; Lang and Fitzhugh 2015). 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 *B. capriscus* (Table 2, Figures 1-2). Two readers independently assigned sex and reproductive state without knowledge of date of capture, 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

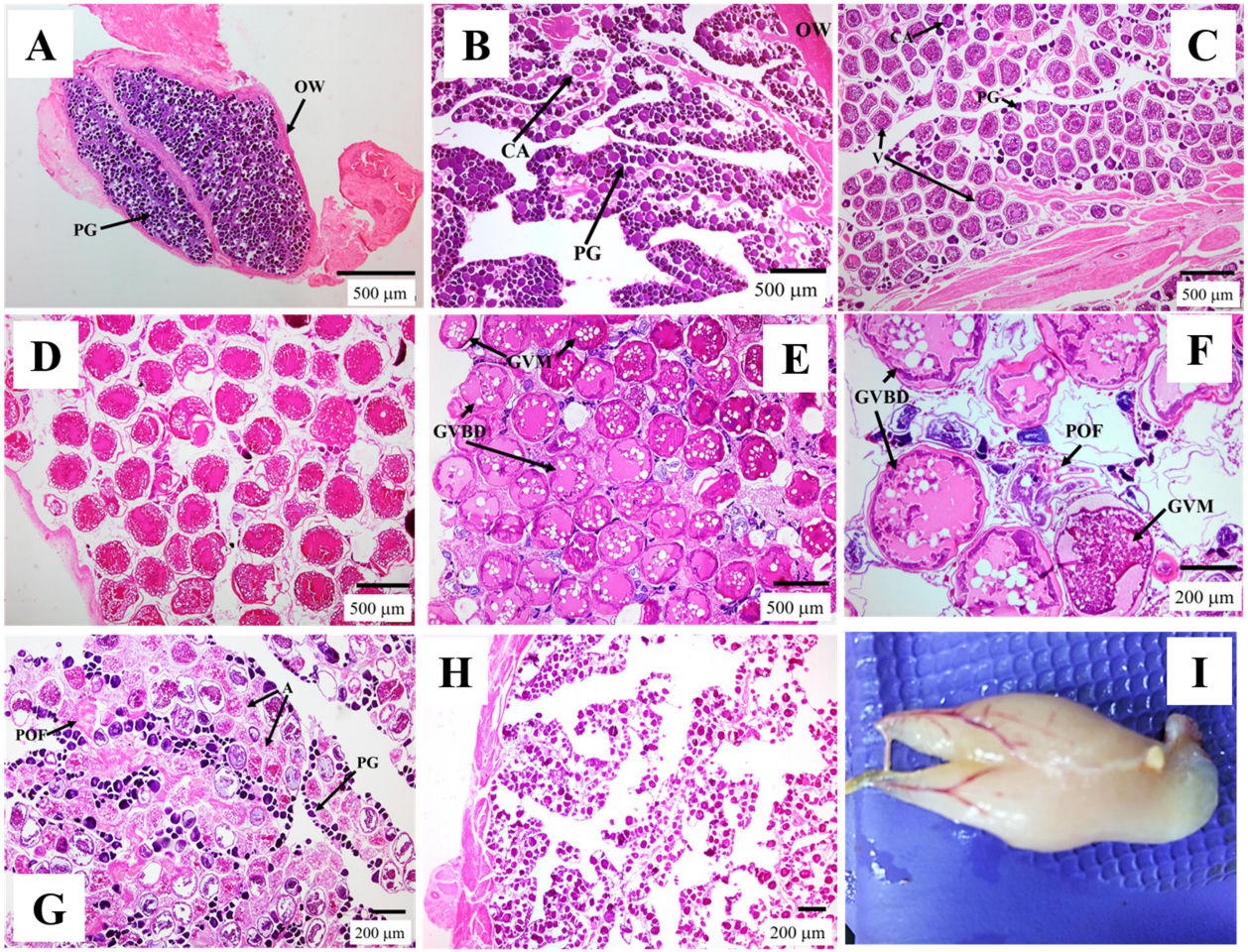

Figure 1. Histological examples of reproductive phases for the gonads of female Queen Triggerfish as described in Table 2: (A) Immature – primary growth oocytes (PG) and thin ovarian wall (OW): (B) Developing (early) – cortical alveolar oocytes (CA); (C) Developing (mid-late) – primary and secondary vitellogenic oocytes present (V); (D) Spawning capable – Oocyte maturation in the most advanced oocytes: zona radiata becomes thin and oocytes are undergoing coalescence of yolk globules; (E, F) Actively spawning subphase – presence of postovulatory follicle complexes (POF) and oocytes show late germinal vesicle migration (GVM) and germinal vesicle breakdown (GVBD); (G) Regressing – more than 50% of vitellogenic oocytes with atresia (A); (H) Regenerating phase PG and thick OW; (I) Whole gonads of a female – note that the two ovaries fuse together in Queen Triggerfish.

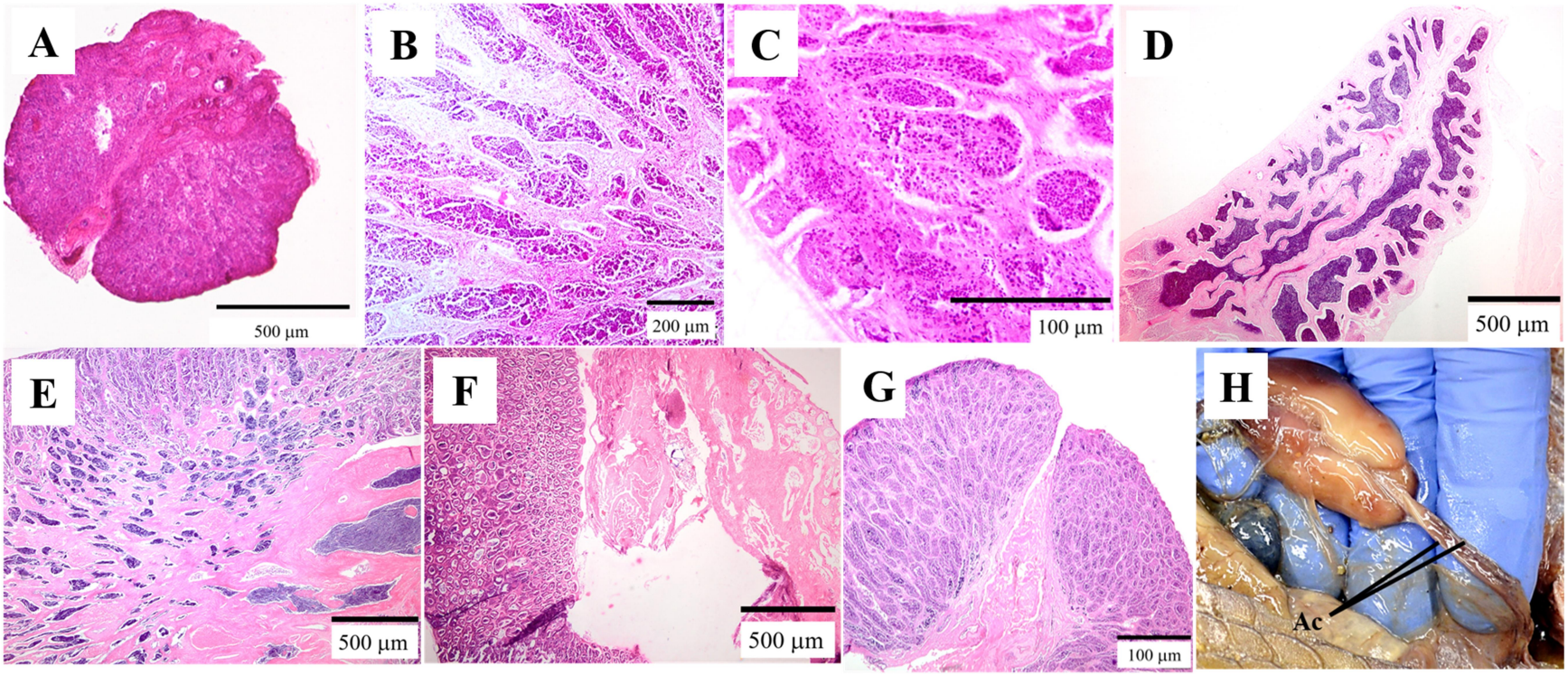

Figure 2. Histological examples of reproductive phases for the gonads of male Queen Triggerfish as described in Table 2: (A) Immature – no spermatocysts with spermatocytes developing in testes; (B) Developing – limited spermatogenesis in testes; elongation of lobules and some development of spermatozoa in testes, but no accumulation in lobules, efferent ducts, and spermatic ducts; (C) Developing – note the development of spermatozoa; (D) Spawning Capable – accessory gland with spermatozoa storage in its expanding ducts; (E) Spawning Capable – large expanded ducts not as densely packed with spermatozoa; (F) Regressing – limited spermatogenesis in testes, some residual spermatozoa in strunken ducts and sinuses; (G) Regenerating – little to no spermatocytes development, empty lobules/ducts and sinuses; (H) Queen Triggerfish male gonad with spermatic duct and accessory glands (Ac).

specimen was eliminated from the analyses. Similar to what we previously observed in the congeneric species *B. capriscus* (Kelly-Stormer et al. 2017), we noted that the gonads of male triggerfish are unique in their structure and function compared to other reef fish species and so we documented the male gonad structure and noted its relevance in assigning reproductive phase for males (Table 1; Figures 2-3).

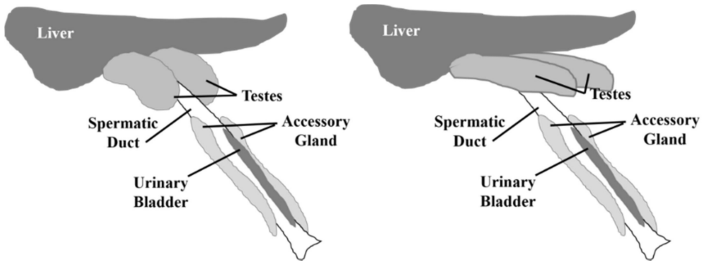

Figure 3. Diagrams of male gonads for Gray Triggerfish (left) and Queen Triggerfish (Right) relative to some of the other internal organs, illustrating the locations of the testes, spermatic duct, accessory gland, and urinary bladder. Note the general differences in testes shape between the species. Both diagrams orginally made by V Shervette. Gray Triggerfish diagram was previously published in Kelly-Stormer et al. 2017.

To qualitatively determine if immature and early developing/regenerating specimens were assigned correctly, we compared the size frequency distributions of fish that were definitely mature (developing, spawning capable, and regressing) to 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 be additional support for correct assignment of phases. Specimens with developing, spawning capable, regressing and regenerating characteristics were considered mature (sexually).

Sex ratios were calculated for PR and STX. Chi-square tests were used to determine if 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 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)] x 100. Mean values for GSI were calculated by month of collection for each sex by island to examine trends in reproduction and spawning as related to the 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 tested separately). 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 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 to mature females was calculated by island for each month in order to determine the months of peak spawning. Spawning fraction, was calculated for each island by determining the proportion of actively spawning females to total number of mature females. Spawning interval was calculated using the 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 if 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. Seasonal increase in secondary oocyte mean diameter, and 4. Atresia is not generalized at the end of the spawning season, and if present it is distributed sparsely along 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 towards a particular oocyte size, counts were made of all oocytes present in 10 microscopic fields using a 4x objective. Oocyte size, obtained by calculating the mean of three measurements of diameter for each oocyte, was only recorded for oocytes sectioned through the nucleus. Measurements ranged from 146-291 oocytes per gonad (mean = 209). Oocyte size frequency histograms were used to assess a hiatus in oocyte development (criteria 1). To determine if mean oocyte diameter increased as the spawning season progressed (criteria 2), we used linear regression. For each of the eight ovary samples, we estimated the day within the spawning season that sample was collected by calculating the number of days between the sample collection date and the full moon in the December prior to the sample collection date. For example, in 2015, the full moon occurred on 25 December, so for a sample collected on 21 July 2016, the number of days between the dates is 209. Spawning season day was then used as the independent variable and mean diameter of secondary oocytes of each sample was the dependent variable for the regression analysis.

RESULTS

Fish collection

Queen Triggerfish sampling occurred from 2013-2018 in waters of Puerto Rico and St. Croix, USVI. A total of 1148 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 fish length (mm FL) and weight (g) for females and males of PR and STX (Figure 4; see Table 5 for length-weight equations). The high coefficient of determination 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: $F_{3,1107} = 2.46$, $p = 0.07$).

Table 3. Overview of depth (m) and size (FL in mm) for males and females including the total number of fish sampled and percentages of males, females, and unknown sex.

Parameter	Puerto Rico	St. Croix
Depth Range (m)	2-90	2-30
Total number of fish	581	567
% Male	55	50
% Female	41	48
% Unknown	4	2
Size Range (mean)	67-434 (291)	190-414 (291)
Male (mean)	67-433 (299)	191-402 (302)
Female (mean)	109-434 (281)	190-414 (277)

Table 4. Results from ANOVA testing for significant differences in mean size (FL mm).

Source	Degrees of Freedom	Mean Square	F	p
Sex	1	127,801	51.042	< 0.001
Island	1	2	0.001	0.980
Island x Sex	1	4790	1.913	0.167
Error	1116	2504		

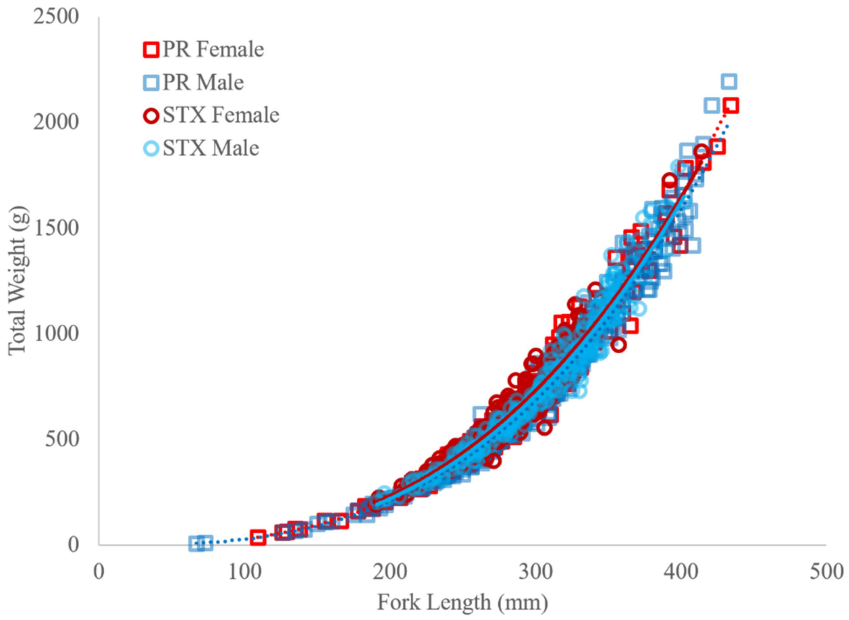

Figure 4. Queen Triggerfish length versus weight for females and males from Puerto Rico and St. Croix.

Table 5. Linear regression equations for length-weight relationship of females and males from PR and STX.

Island/Sex	Equation	R^2
PR Female	$y = 5 * 10^{-5} x^{2.9018}$	0.99
PR Male	$y = 4 * 10^{-5} x^{2.9231}$	0.99
STX Female	$y = 7 * 10^{-5} x^{2.8238}$	0.99
STX Male	$y = 5 * 10^{-5} x^{2.8892}$	0.97

The overall size frequency distributions between males and females (islands combined) were significantly different, with a larger proportion of males in the larger size classes (K-S: $Z = 3.84$, $p < 0.001$; Figure 5; Table 6). The size frequency distributions of males and females were also significantly and similarly different in PR ($Z = 1.77$, $p = 0.004$) and in STX ($Z = 3.97$, $p < 0.001$).

Reproduction

A total of 1138 gonads were collected. Sex and reproductive phase were assigned to 1120 (98%) Queen Triggerfish. In general for many fish species, the anatomy of the male and female gonads are relatively similar in that they consist of two lobes that are posteriorly attached and release gametes through the oviduct for females and the spermatic duct males. Female triggerfish gonads are similar in shape compared to other fish species (Kelly-Stormer et al. 2017). For male Queen Triggerfish, we documented that the gonad anatomy and structure was similar to Gray Triggerfish (Kelly-Stormer et al. 2017); the male gonads consist of testes, spermatic duct, and accessory glands (Figures 2-3). The accessory glands are important because they store spermatozoa before spawning. One minor difference between male Queen and Gray Triggerfish was that the testes are more elongate in Queen Triggerfish compared to the more kidney-bean shaped testes in Gray Triggerfish (Figure 3). 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 are 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,

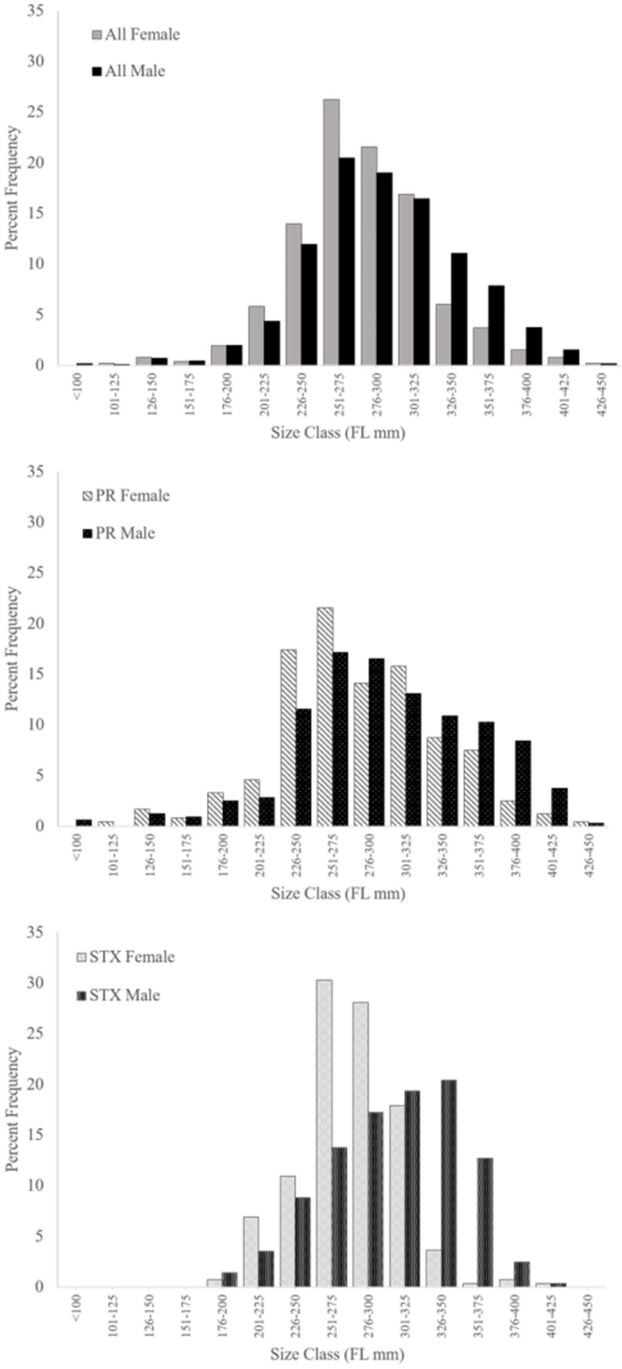

Figure 5. Size class distributions between females and males for islands combined, PR samples, and STX samples.

Table 6. Results of Kolmogorov-Smirnov tests for differences in the size frequency distributions between males and female by islands.

Comparison	Z-statistic	P
Overall Female vs Male	3.84	< 0.001
PR Female vs Male	1.77	0.004
STX Female vs Male	3.97	< 0.001

spawning capable and regressing) and early developing/regenerating specimens and (2) the minimal overlap in histograms for immature and early developing/regenerating specimens (Figure 6).

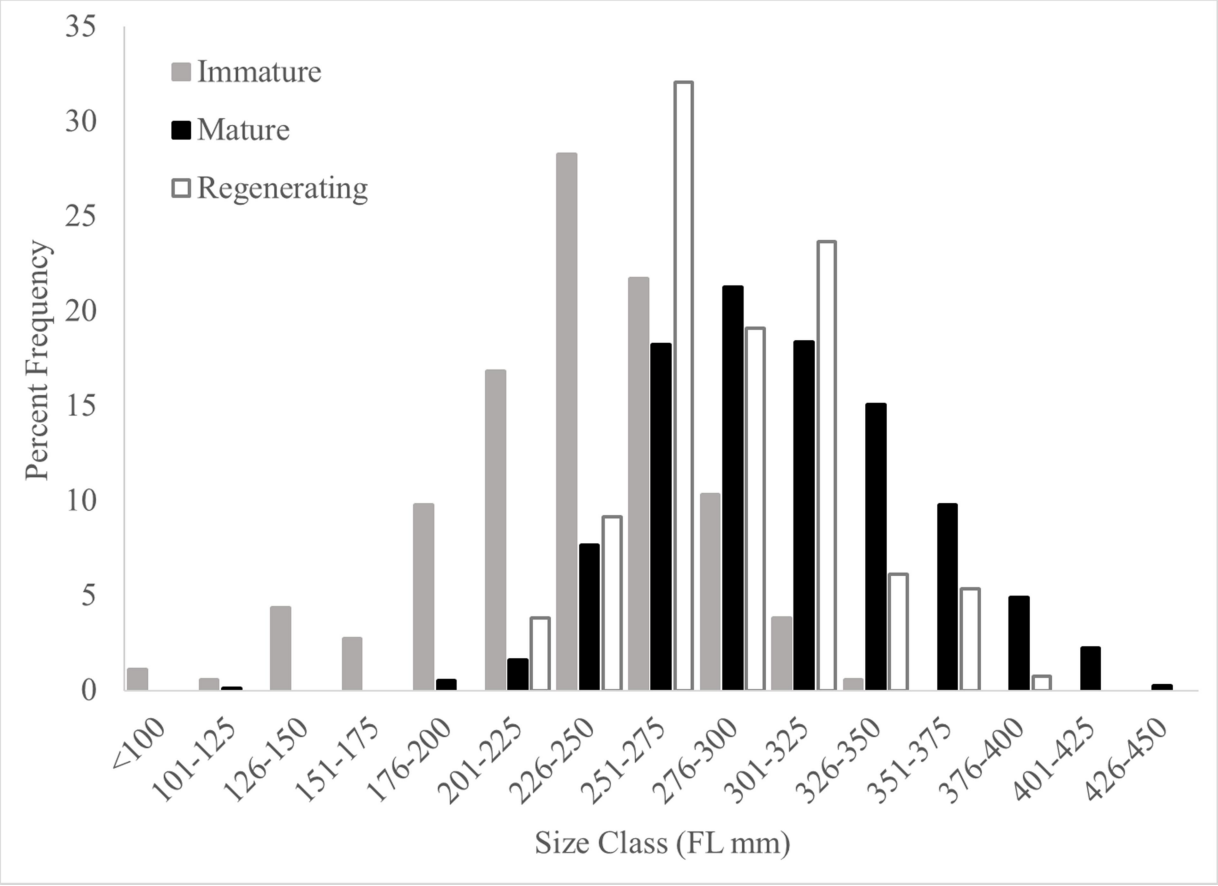

Figure 6. Size frequency distributions of female and male Queen Triggerfish with gonads categorized as immature, mature (developing, spawning capable, regressing), or regenerating.

The overall female:male sex ratio for samples from PR was 1:1.3, which differed from the expected 1:1 ratio ($\chi^2 = 11.4$, df = 1, P = 0.001). In STX samples, sex ratio was not significantly different from the 1:1 ratio ($\chi^2 = 0.2$, 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). Male size at 50% maturity was 206 mm FL (Table 7, Figure 7). The smallest mature female was 215 mm FL and the largest immature female was 285 mm FL. Female size at 50% maturity was 256 mm FL (95% CI = 246-264 mm), and all females larger than 276-300 mm FL were sexually mature (Table 7, Figure 7).

For STX samples, the smallest mature male was 184 mm FL, and the largest immature male was 253 mm FL (Table 7). Male size at 50% maturity was 211 mm FL, and all males larger than 251-275 mm FL were mature (Table 7, Figure 7). The smallest mature female was 219 mm FL, and the largest immature female was 329 mm FL. Female size at 50% maturity was 245 mm FL, and all females were mature by 351-375 mm FL (Figure 7).

Table 7. Sizes at sexual maturity for Queen Triggerfish males and females from PR and STX.

Sex	Immature Max Size (mm FL)	Mature Min Size (mm FL)	L_{50} (95% CI)
PR			
Female	285	215	256 (246-264)
Male	230	196	206 (180-218)
STX			
Female	329	219	245 (238-251)
Male	253	184	211 (196-220)

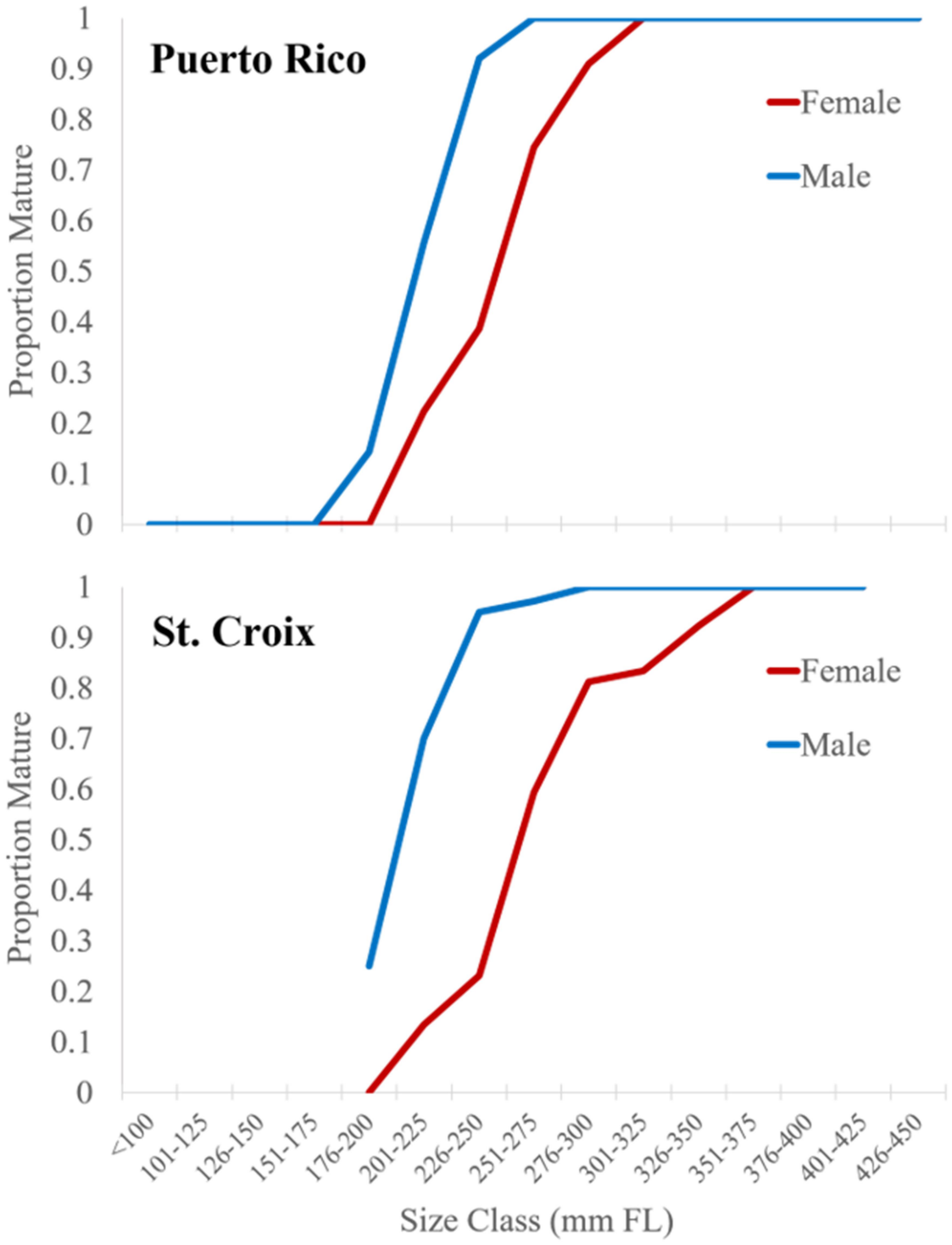

Figure 7. Female and male 50% sexual maturity curves for Puerto Rico and St. Croix.

Monthly GSI was calculated separately for females and males of PR and STX (Figure 8). 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). Pair-wise, 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 8). 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 8). For males, a significant difference in mean monthly GSI among months occurred for PR ($p < 0.001$) and for STX ($p < 0.001$; 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 8). 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 8). The monthly percent of spawning capable Queen Triggerfish samples peaked in PR during December and January and in STX during January and February (Table 9).

Based on the PR dataset from 2013-2018, the beginning of the spawning season was 24 December, which was the earliest date that oocyte maturation was observed in females in any year. The end of the spawning season was 22 August, which was the latest date that late-developing oocytes and POCs occurred in females observed in any year. This results in a PR spawning season of 241 days (Figure 9). Based on the STX dataset from 2015-2018, the earliest date that oocyte maturation was observed in females of any month was 11 December, and the

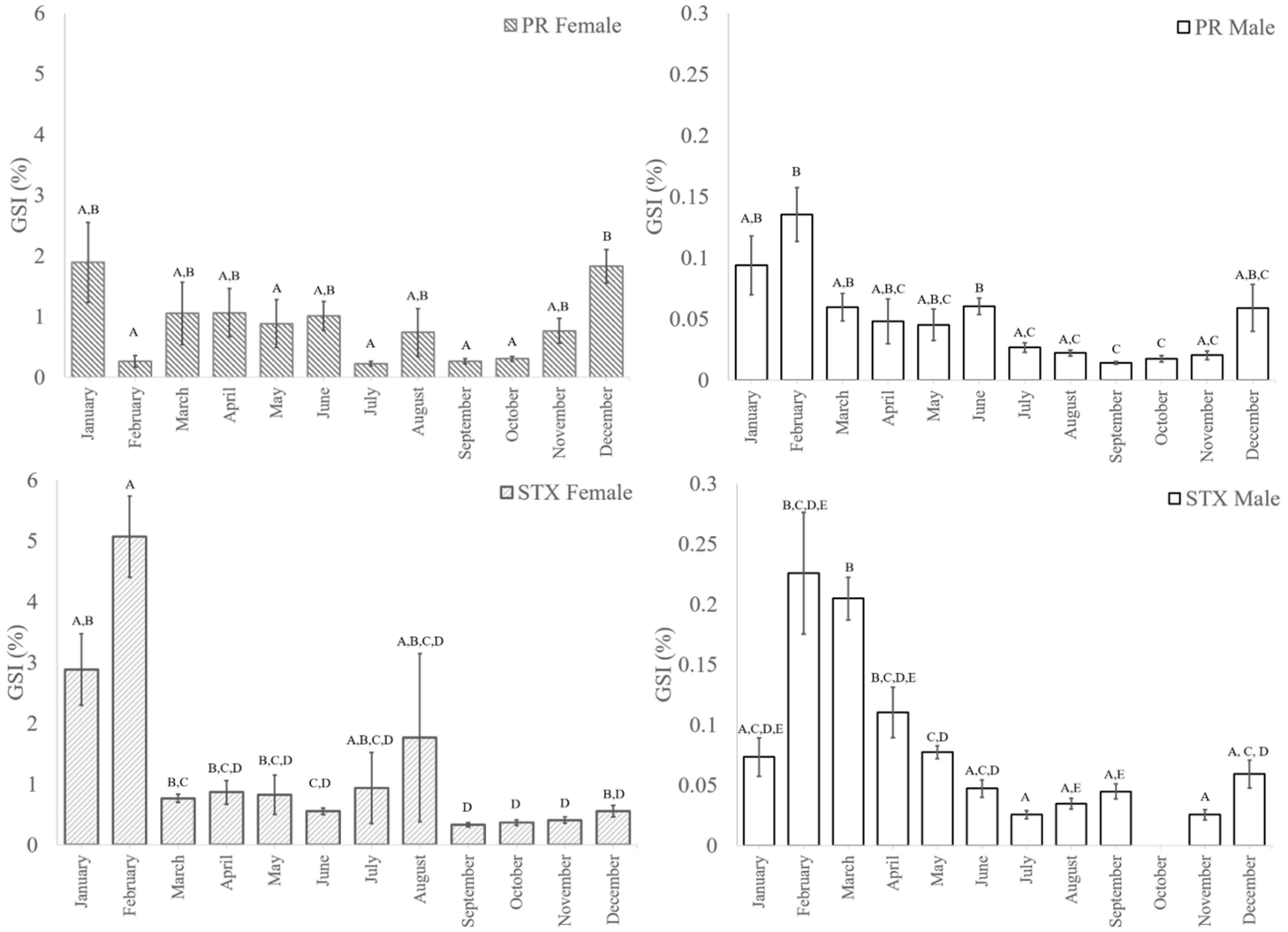

Figure 8. Mean monthly GSI for sexually mature Queen Triggerfish females and males from Puerto Rico and St. Croix. Error bars represent standard error. Letters within each graph indicate significant differences in monthly mean GSI for pairwise comparisons (α = 0.05). Note that no mean value is presented for STX Males; this is because the scale for weighing gonads that measures to the 0.01 g malfunctioned before testes could be weighed.

Table 8. ANOVA results for GSI by month

Island and Sex	Source	Degrees of Freedom	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		

Table 9. Percent of female Queen Triggerfish in spawning capable phase (# of spawners/# of mature females sampled) for each month they occurred.

Month	Puerto Rico	St. Croix
December	33% (3/9)	5% (1/19)
January	21% (5/24)	35% (10/29)
February	-	73% (8/11)
March	11% (2/18)	5% (1/19)
April	7% (1/14)	6% (2/34)
May	7% (1/15)	8% (1/13)
June	21% (4/19)	-
July	-	6% (1/15)
August	4% (1/23)	14% (1/7)

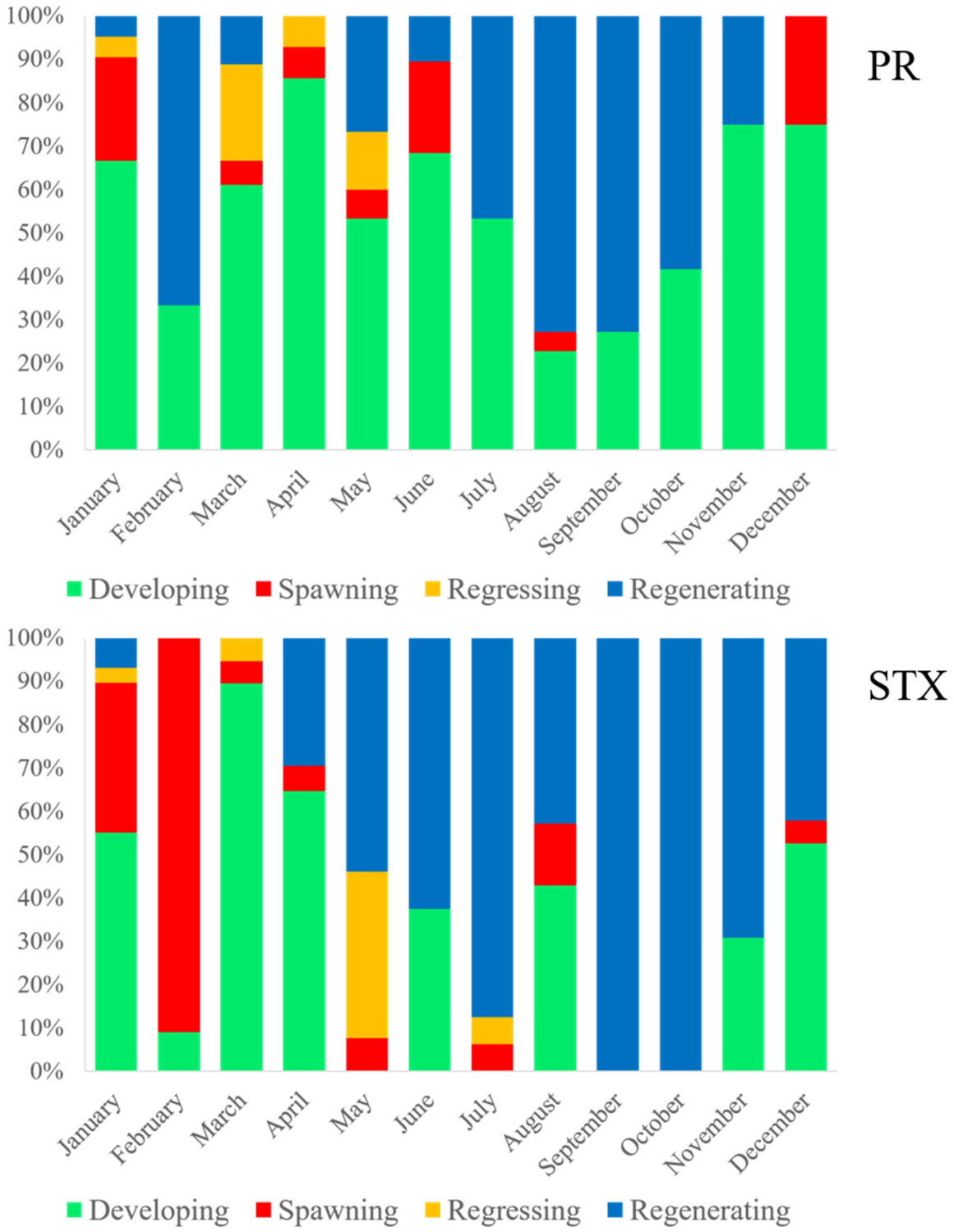

Figure 9. Female Queen Triggerfish reproductive seasonality. Monthly proportions of individual females in each reproductive phase.

end of the spawning season, the latest date of late developing oocytes, was 9 August. This yields an STX spawning season of 241 days (Figure 9). However, STX fishers reported observing nest guarding by female Queen Triggerfish as early as the week after of the full moon in December (V. Shervette, unpublished data) and as late as the week after the full moon in August. If Queen Triggerfish time spawning events around the full moon starting in December and continue to spawn through the full moon in August, then that yields a spawning season of a maximum of 267 days. Spawning capable males occurred in relatively high proportions every month in PR and STX (Figure 10).

Only three females in PR and three in STX had gonads with hydrated oocytes/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 days (or 1/0.02, the reciprocal of the overall proportion of spawning females expressed in days) and for STX females was approximately 56 days. With a spawning season ranging from 241-267 days in PR and STX, a female Queen Triggerfish can potentially spawn 4-5 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 11), all showing a clear hiatus between sizes of oocytes in the cortical aveolar stage and the vitellogenic stage (Figure 11). This is indicative of a group-synchronous pattern of oocyte development, and normally 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 does not meet the criteria for determinate fecundity in Queen Triggerfish.

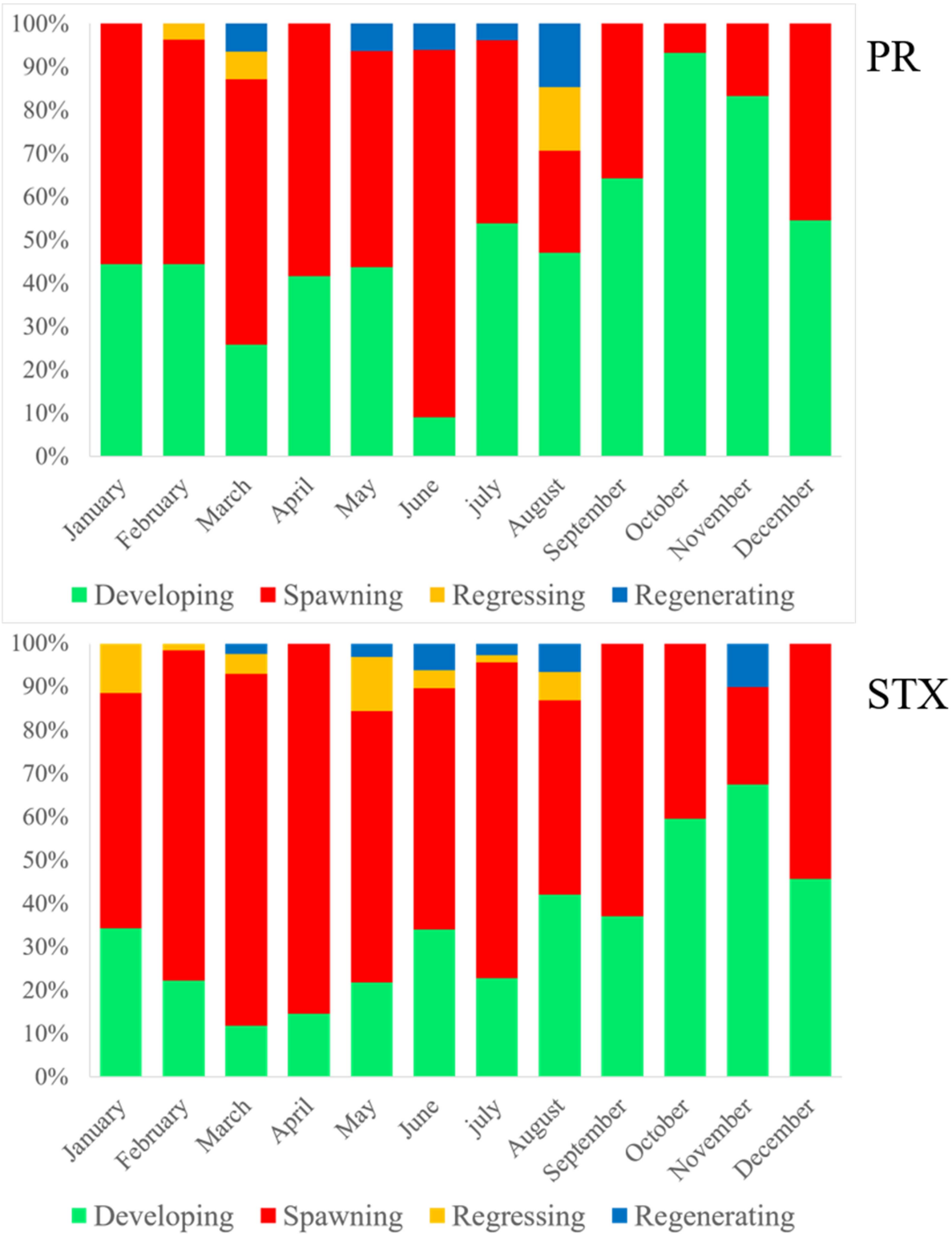

Figure 10. Male Queen Triggerfish reproductive seasonality. Monthly proportions of individual males in each reproductive phase.

Table 10. Spawning fraction, spawning interval, and spawning frequency of females from Puerto Rico and St. Croix. The number of calendar days from the full moon of December to the full moon of August is 267. In PR, the first spawning capable female occurred on 24 December and the last spawning capable female occurred on 22 August, yielding a total of 241 days. In STX, the first spawning capable female occurred on 11 December and the last spawning capable female occurred on 9 August, yielding a total of 241 days.

Metric	Puerto Rico	St. Croix
Spawning Fraction	0.02 (3/164)	0.02 (3/169)
Spawning Interval	54	55
Spawning Frequency	4-5 times/y	4-5 times/y

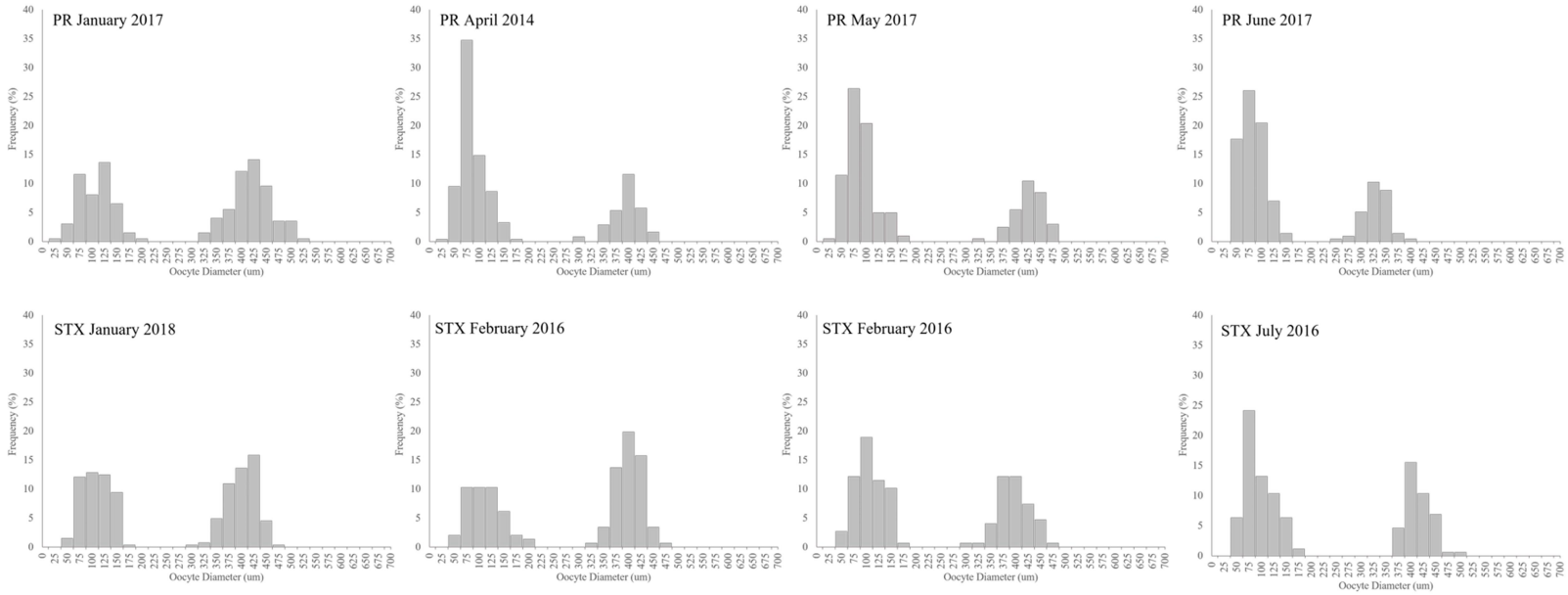

Figure 11. Size frequency distributions of oocyte diameter from histological sections of eight Queen Triggerfish females.

DISCUSSION

The current 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 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; Kawase 2003; Kuwamura 1997; Simmons and Szedlmayer 2012). The mean length of Queen Triggerfish males was significantly larger than females for PR and STX. Similar findings have been reported for Gray Triggerfish populations, in which males are significantly larger than females, attain 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 sizes 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 Crosshatch Triggerfish *Xanthichthys mento* establish and defend territories before spawning and during egg care around the Izu Islands of Japan. Females nesting in a male's territory focus 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.

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 mean diameter of vitellogenic oocytes did not increase as the spawning season progressed. Lang and Fitzhugh (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 (Ganias et al. 2004; Nakazono 1993; Yoneda et al. 1998) including the Spiny Damselfish *Acanthochromis polyacanthus*, another demersal egg-laying and brood-caring reef fish (Nakazono 1993). Male and female Spiny Damselfish 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 female Spiny Damselfish 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 and Gray 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 fish fisheries species that are pelagic spawners, but potentially 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 seem associated with the full moon. Spawning activities associated

with a specific lunar phase have been documented in other Caribbean reef fish species including 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. STX spear-fishers reported observing Queen Triggerfish guarding shallow nests for up to a week after the full moon in December 2017, January 2018, February 2018, and March 2018. Lunar-cycle-driven spawning activities have been observed in other triggerfish species (Donaldson and Dimalanta 2011; Gladstone 1994). Future Queen Triggerfish sampling efforts are needed to further investigate and confirm for both islands spawning activities as they relate to the lunar monthly cycle.

Only one main study examined the reproductive biology of Queen Triggerfish in the Caribbean. Aiken (1983) obtained fisheries-independent Queen Triggerfish samples in 1969-1973 from waters of Jamaica. That study combined the macroscopic observations of gonads from males and females and reported that the reproductive season for Queen Triggerfish was January-March, May, July-December. Our study does not support those initial findings (Figure 10). 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). 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 each year during the month of February, due to recurring current and wind patterns that prevented fishers from fishing; over 2013-2018 we only

managed to collect four mature females for February (Table 10). However, spawning capable females did occur at a high proportion of 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 (Bernardes and Dias 2000; Kacem and Neifar 2014; Kelly-Stormer et al. 2017; Lang and Fitzhugh 2015; Ofori-Danson 1990). Length of 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. Queen and Gray Triggerfish are two difference species. 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 (Ganias 2009; Hunter et al. 1992; Murua and Saborido-Rey 2003). We estimated that female Queen Triggerfish could spawn 4-5 times throughout the spawning season (Table 11), which is half the spawning frequency estimated for Gray Triggerfish (Kelly-Stormer et al. 2017; Lang and Fitzhugh 2015). Queen Triggerfish and Gray Triggerfish share a relatively unique reproductive strategy compared to other medium- to large-bodied fisheries 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 to the larvae of pelagic spawners. Additional research on Queen Triggerfish larvae 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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