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Oogenesis and fecundity type of Gray Triggerfish (*Balistes capriscus*) in the Gulf of Mexico

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Abstract The fecundity of Gray Triggerfish (*Balistes capriscus*) has been difficult to estimate as few imminent and recent spawning females have been detected. Our study focused on verifying the pattern of oogenesis and fecundity type. From 1999-2012, 1092 female Gray Triggerfish were collected from the eastern Gulf of Mexico with subsets used to calculate condition indices and assess ovarian histology. Gonadosomatic and hepatosomatic indices and Fulton's condition factor indicated liver and somatic energy stores increased prior to spawning and were depleted throughout the spawning period. This is characteristic of a capital pattern of energy storage and allocation to reproduction. Typical of a capital pattern, we observed a hiatus in oocyte size distribution and group synchronous oogenesis, both traits of a deterministic fecundity type. However, evidence that fecundity was not set prior to spawning included the observation of "de novo" vitellogenesis during the spawning season; sec ondary oocytes increased in number and failed to increase in mean size over time. Thus Gray Triggerfish exhibit an indeterminate fecundity type with mixed reproductive traits that may characterize species exhibiting female parental care in warm water environments. Further, we estimated secondary oocyte growth (37 µm/d) based upon the timelag of postovulatory degeneration. Using oocyte growth rate and the proportion of females bearing postovulatory follicles, the interspawning interval was estimated to range from 8 to11 d. This indicates production of 8-11 batches per female may occur during an estimated 86 d reproductive period. The hiatus in oocyte size distribution was used to define a minimum size (250 µm) from which to distinguish an advancing batch of secondary growth oocytes. Batch fecundity (BF) ranged from 0.34 to 2.0 million eggs and was significantly related to fork length (FL): BF=8,703.69*FL(mm) – 1,776,483 (r^2=0.56).

Introduction

Gray Triggerfish (*Balistes capriscus*) is an economically important species in both recreational and commercial fisheries of the Gulf of Mexico (Gulf). Like other Gulf species, measures of reproductive potential are needed for assessment of stock status, and those estimated from fecundity metrics are more accurate (Paulik 1973, Tomkiewicz et al. 2003, Lambert 2008, McBride et al. 2015). Unfortunately, particular fecundity methodologies have been applied for some species without validation, necessitating corrections (e.g., Arocha 2002, Gordo et al. 2008, Fitzhugh et al 2012). Understanding oogenesis allows classification of the fecundity type and provides insight on spawning frequency (Murua and Saborido-Rey 2003, Witthames et al. 2009).

Although information on Gray Triggerfish reproduction exists in research reports, its pattern of oogenesis and fecundity type has not been validated. In a 2005 Gulf stock assessment, Gray Triggerfish is assumed to be an asynchronous indeterminate spawner producing numerous batches (Ingram 2001, SEDAR 9 2006). Previous unpublished studies had difficulties determining Gray Triggerfish fecundity and/or spawning frequency (Wilson et al. 1995, Hood and Johnson 1997, Moore 2001, Ingram 2001), primarily because females with advanced stage oocytes (oocyte maturation) were very rare among samples collected and postovulatory follicles were detected in few females (Ofori-Danson 1990, Wilson et al. 1995, Hood and Johnson 1997, Ingram 2001, Moore 2001). This has resulted in uncertainty and a broad range in estimates of spawning frequency.

Part of the difficulty in classifying reproductive traits and fecundity type may lie in the rather unique biology of Gray Triggerfish. It is a nesting species with a haremic mating system wherein both sexes share short term parental care (Simmons and Szedlmayer 2012). Considering the mating system of Gray Triggerfish, a better understanding of fecundity type may depend on determining the female reproductive energy strategy utilized from the income-capital breeding typology (Stearns 1992, Jonsson 1997, Jager et al. 2008, McBride et al. 2015). Capital breeders are commonly coldwater species experiencing dynamic seasonality. Energy for reproduction is acquired and stored when food may be abundant before the reproductive season begins (Jager et al. 2008). In income breeders, a rapid transfer of energy to reproduction may occur when

ecosystem productivity is high yet timing of productivity may be less predictable in time and space (Santos et al. 2010, McBride et al. 2015). Species also may exhibit mixed capital and income traits depending on the degree of available surplus energy and demands from activities such as migration and parental care (Jager et al. 2008, McBride et al. 2015). The accessory behaviors of parental care have been associated with high energy demands in several species of fishes and can affect other life history traits (Kuwamura 1997, Donelson et al. 2008, Jager et al. 2008).

The objectives of this study were to verify fecundity type, classify energy storage in light of the mating system, calculate spawning frequency, and postulate a method to estimate annual fecundity for Gray Triggerfish in the Gulf of Mexico.

Methods

Sample collection and condition indices

In an effort to observe a time series of reproductive development, hook-and-line sampling targeting Gray Triggerfish was conducted May-July 2012 offshore Panama City, Florida. All captured specimens were kept, measured to the nearest mm of fork length (FL) and weighed to the nearest gram (g). Dorsal spines were extracted for aging; livers were excised and weighed; gonads were removed, macroscopically assessed for sex and reproductive state, weighed; and ovaries were fixed in 10% neutral buffered formalin (<24h on ice) for further processing in the laboratory (see below). Length, weight, macroscopic sex and reproductive state, and in some cases, gonad and liver weight and ovary tissue samples, were also measured or obtained from specimens collected in the northern Gulf, 1999-2012, by trap, spear, and hook-and-line in various fishery-independent and -dependent surveys (Table 1). Gonadosomatic index (GSI),

$$GSI = \left[\frac{Gonad\ Weight}{Total\ Weight - \ Gonad\ Weight}\right] * 100$$

hepatosomatic index (HSI),

$$HSI = \left[\frac{Liver\ Weight}{Total\ Weight - \ Liver\ Weight}\right] * 100$$

and Fulton’s condition factor (K; Ricker 1975)

$$K = 100 * \frac{Total\ Weight}{(Fork\ Length)^3}$$

based upon weight in g and length in cm were used to examine the relationship between energy storage and reproduction.

Oocyte staging and measurements

Oocyte stage and diameter were used to determine fecundity type in Gray Triggerfish as determinate or indeterminate. The key criteria used to test for determinacy followed Hunter et al. (1992) and Murua and Saborido-Rey (2003), which include 1) a hiatus in size distribution of developing oocytes, 2) increase in secondary oocyte diameter through the spawning season, 3) decrease in number of secondary growth oocytes through the season, and 4) secondary oocyte growth rate that does not allow for “de novo” vitellogenesis within one spawning period.

To obtain oocyte stage and size data, one ~150 mg subsample was removed from the posterior region of all available ovaries following Harris et al. (2002), weighed, and oocytes were disassociated with forceps and plated as a whole mount in a petri dish (150 mm diameter) to be imaged by an EPSON V750 scanner. The top or cover of the scanner rested on the petri dish parallel with the bottom of the scanner. The scanner’s software (EPSON Scan) was set to scan “Positive Film” at 2400 dpi in “Professional” mode, which allowed for a transmitted light image with high resolution.

This image was then uploaded into Object J, a plugin for ImageJ (ver. 1.47s), for image analysis. Primary growth oocytes were difficult to count and measure in the scans due to their small size (<100 µm) and low degree of clarity (Figure 1). For 2012 samples, secondary growth oocytes,

including cortical alveolar and vitellogenic oocytes, were automatically counted and measured using a macro within the ObjectJ plugin (Figure 1; http://simon.bio.uva.nl/objectj/7a-Examples.html). Counts of secondary oocytes were used to estimate batch fecundity based upon an oocyte size criterion (see results) and expressed as somatic relative fecundity (eggs g^{-1} ovary-free body weight, Kjesbu et al. 1998) and oocyte density (secondary oocyte count g^{-1} ovary weight) for comparison with other studies.

Oocyte size frequency histograms were used to assess a hiatus in oocyte development while oocyte enumeration and diameter was regressed against time within the spawning season to resolve criteria two and three. All regressions were performed using the R statistical software package (ver 2.13.1).

Postovulatory follicle measurements

Additionally, ovary samples from all years were processed histologically with H & E stain. Pictures were taken with an ocular microscope camera (MiniVid; 5MP) of each histology slide with postovulatory follicles (POF) present. Five random images were chosen from all possible non-repetitive images of each histology slide with oocytes filling the frame. Each oocyte that represented the leading stage of oogenesis (cortical alveolar or vitellogenic) and was sectioned through the germinal vesicle was measured for diameter (Foucher and Beamish 1980). The area of every POF present within the five random images was measured and plotted as a predictor of leading oocyte stage diameter.

Data analysis

Oocyte growth was determined using methods similar to Ganias et al. (2011). Postovulatory follicle area was converted to a percentage of POF duration using known POF durations from oceanic species with a similar temperature regime (Jackson et al. 2006). Simple linear regression was used to investigate changes in oocyte size, number, and growth over time.

The secondary oocyte growth rate (G) was calculated using the difference in oocyte size (O_i) after some period of growth (timelag, t_i) and average size of oocytes from the spawning batch at the beginning of the spawning batch development for the whole population ($\beta_\circ$; Ganias et al. 2011). The average size of oocytes at the beginning of the spawning cycle were observed in fish with the most recent POFs. As POFs are resorbed relatively quickly in Gulf warm water fishes, a time lag (t_i) could be estimated from the percent change in the POF area over an expected 24 hour duration (≤24 h in 24-28.5 C water, Jackson et al. 2006).

$$G = \frac{O_{\mathrm{i}} - \beta_\circ}{t_{\mathrm{i}}}$$

Spawning frequency was calculated using both ISI (Ganias et al. 2011) and POF (Parker 1980) methods. The ISI method assumes the secondary oocyte growth is group synchronous and that the growth rate is constant, thus the ISI is the difference between minimum (O_b) and maximum (O_e) observed size of secondary oocytes divided by the growth rate (G).

$$ISI = \frac{O_{\mathrm{e}} - O_{\mathrm{b}}}{G}$$

The POF method is based upon the proportion of females bearing postovulatory follicles respective to the total number of mature females sampled within the spawning season. Maturity was based upon the presence of vitellogenic oocytes. Since we estimate POF duration to be 24 h, the inverse of the proportion of females bearing POFs yields the expected interval in days between spawning (Parker 1980). Due to the often opportunistic sample collection during surveys, sample sizes varied year-to-year and were ad hoc with respect to the timing of reproductive development and spawning. Samples were grouped across years to increase sample size for the POF method. This grouping necessarily assumes there is no difference in spawning intensity, hence prevalence of POFs, among years.

Results

Field sampling and condition indices

From 1999 through 2012, n=1092 Gray Triggerfish females were collected in the northern Gulf of Mexico from various fishery independent and dependent sources with 85% captured from 10-50 m depth (Table 1). Of those, 629 had gonad weight data to calculate GSI, 238 had liver weight to estimate HSI, and 531 were histologically examined for POFs. Hepatosomatic index peaked in May, a month before peak spawning (peak GSI), decreased sharply from May to September, and then began to increase again (Figure 2). This pattern is consistent with a translocation of energy from the liver to the gonad during May-August with a replenishment of liver energy stores beginning in September and continuing until spawning resumed the following year. Seasonally, Fulton's condition factor from all 1092 females decreased after June, consistent with the HSI and GSI evidence that energy stores increase during the spring, are highest at the onset of spawning and are depleted throughout the spawning period.(Figure 3).

Oocyte size analysis

Examination of oocyte size frequency distribution data suggested a group synchronous pattern of oocyte development commonly associated with determinate fecundity. Three randomly selected females were chosen from each month, May through July (total of 9 females) to represent oocyte size distribution (Figure 4). The individual oocyte size frequencies in seven of the randomly selected females were bimodally distributed, and four of these seven showed a clear hiatus between the cortical alveoli and vitellogenic oocyte stages. Those females with a unimodal peak in oocyte distribution had smaller oocytes on average with early vitellogenic oocytes that we expect would have disassociated in size from primary growth and cortical alveoli stages with time. However, linear regressions of secondary oocyte diameter (r^2=0.05, P=0.148; Figure 5) and number of secondary oocytes (r^2=0.01, P=0.491; Figure 6) versus time during the spawning season were not significant. Secondary oocytes did not increase in diameter and did not decrease in number as the spawning season progressed, which, contrary to the oocyte size frequency distribution results, does not support a determinate type.

Oocyte growth and interspawning interval

Despite more intensive sampling during the spawning season in some years (e.g., 2012), POFs were rarely found, occurring in only 31 of the 531 females sampled for histology. The majority of these 31 recently spawned females possessed POFs with a collapsed lumen, and thus a smaller surface area and a skewed size distribution. However, within 2001 the POF surface area data for seven recently spawned females were tested and fulfilled linear regression assumptions for normality among means (Shapiro-Wilk normality test: W=0.8957, P=0.306). A Shapiro-Wilk normality test also indicated that secondary growth oocyte diameter was normally distributed (W=0.9288, P=0.541). Secondary oocyte growth exhibited an inverse linear trend with POFs; as secondary oocytes grew larger, POFs diminished in surface area (r^2=0.63, P=0.034; Figure 7).

By estimating the growth rate of secondary oocytes (G), the interspawning interval was calculated to be 11 days. This computation uses the POF duration from oceanic fish species that reside in similar water temperatures (Jackson et al. 2006) and the POF "clock" to estimate the time lag (t_i). We found that maximum surface area of a Gray Triggerfish postovulatory follicle was 12 µm^2. Understanding that a POF shrinks as it ages and may last about 24 h (1 d), we observed a 74% decrease in POF size during a oocyte size regression series from 2001 (Figure 7). Thus the proportional decrease in POF size over time serves as the denominator to standardize to 24 h (1 d) the corresponding growth in secondary oocytes; estimated at 37 µm/d. The resulting ISI of 11 days is rapid enough to allow for "de novo" vitellogenesis to occur multiple times during the spawning season, which indicates an indeterminate fecundity type. Therefore, three of the four hypothesis tests support indeterminate fecundity in Gray Triggerfish.

Using the POF method, the average interspawning interval was calculated to be 7.6 d. Females were observed on 43 sampling dates between 26 May and 19 August from years 1999 through 2012. The spawning interval was calculated as the inverse of the proportion of females with POFs (n=31) among the total number of mature females (n =236). Thus the proportion (31/236) with POFs is 0.131 and the inverse is 7.6. For the 86 d spawning season, estimated as the interval during which POFs were detected, the POF and ISI methods predicted up to 8 batches and 11 batches per season, respectively.

Batch fecundity estimation

Gray Triggerfish undergoing oocyte maturation (OM) and/or hydration were rarely collected during routine sampling, adding to the challenges to estimating fecundity. Of the 236 maturing females collected during the spawning season only one was a ripe female undergoing oocyte maturation. Because of the group synchronous development, the population of advancing vitellogenic oocytes may be considered equivalent to a batch. However, all fish with POFs did possess cortical alveolar and newly formed vitellogenic oocytes with diameters <250 μm (Figure 7). Therefore, in order to distinguish the oldest batch, only secondary oocytes ≥250 μm were counted.

Batch fecundity ranged from 0.34 to ~2.0[1.99] million eggs and somatic relative fecundity ranged from 590 to 2,686 eggs per gram of ovary-free body weight in specimens 266-386 mm FL (Table 2). A portion (56%) of the variance in batch fecundity (BF) was significantly explained by fork length (FL; BF=8,703.69*FL(mm)-1,776,483; r^2=0.56, P<0.0001; Figure 8).

Discussion

Similar to balistids world-wide, Gray Triggerfish in the Gulf spawn in pairs, are nest builders that establish and defend territories, and guard their eggs (Kuwamura 1997, Kawase 2003, Simmons and Szedlmayer 2012). Perhaps related to this reproductive strategy, Gray Triggerfish exhibit group-synchronous secondary oocyte development, unusual for warm-water species. Wallace and Selman (1981) defined group synchronous development as the presence of two populations of oocytes clearly distinguished from one another; a larger more synchronous population and a smaller more heterogeneous one. Gray Triggerfish clearly exhibited this pattern with a distinct size hiatus between cortical alveolar and vitellogenic stage oocytes.

Group synchronous oocyte development pattern is typically associated with determinate fecundity (Murua and Saborido-Rey 2003, McBride et al. 2015), however we found evidence that Gray Triggerfish are indeterminate. The number of vitellogenic oocytes did not decrease while the diameter did not increase through the spawning season. Both of these results suggested

that "de novo" vitellogenesis was occurring, thus violating the criteria of a determinate fecundity type. While rare in the literature, other species have exhibited both group synchronous oocyte development and the potential for de novo vitellogenesis; Spiny Damselfish (*Acanthochromis polyacanthus*), Mediterranean Sardine (*Sardina pilchardus sardine*), and Anglerfish (*Lophius litulon*), (Nakazono 1993, Ganias et al. 2004 and Yoneda et al. 1998).

Perhaps most diagnostic, we estimated that growth rate of secondary oocytes (37 µm/d) was rapid enough to yield multiple batches within an estimated 86 d spawning season. While there are few published values of secondary oocyte growth rate, some comparisons are available from higher latitude fishes. For two species from the British Isles, rates of ~3.2 µm/d for sole (*Solea solea*) (based upon increase in mean diameter over 143 d; Witthames and Greer-Walker 1995) and ~1.3 µm/d for Atlantic mackerel (*Scomber scombrus*) (Greer-Walker et al. 1994) were reported. In both cases, secondary oocyte development proceeded slowly enough that de novo vitellogenesis could not occur within the spawning season; hence, the fecundity type was considered determinate.

Gray Triggerfish exhibited traits that are common in capital breeding species. Condition indices (HSI, K) peaked just before or close to the onset of spawning followed by declines through the reproductive period similar to other species with a capital pattern (Alonso-Fernández and Saborido-Rey 2012). As well, HSI exceeded GSI in all months which emphasizes the importance of the liver for energy storage and mobilization (Htun-Han 1978, Rinchard and Kestemont 2003). In more extreme capital species, the seasonal increase in condition indices may occur well outside of the reproductive period (e.g., Htun-Han 1978, Yoneda et al. 2001, Alonso-Fernández and Saborido-Rey 2012). By contrast income breeding species lack or exhibit weak seasonal patterns in condition indices (e.g, Domínguez-Petit et al. 2010). While we postulate that Gray Triggerfish are capital breeders, we cannot reject the possibility that food intake during the spawning season may partially provide the energy needed for egg production. However, the Gray Triggerfish liver seems to be important in energy mobilization and may help to insure that oocyte development proceeds even if energy intake is low (e.g., Allen and Wootton 1982, Alonso-Fernández and Saborido-Rey 2012) or where there is a hiatus in feeding during parental care.

We find that Gray Triggerfish in the Gulf show attributes of indeterminate fecundity associated with warm water environments. Yet the species also exhibits group synchronous oocyte development and a capital breeding pattern associated with territoriality and female parental care, traits most often linked with determinate fecundity (McBride et al. 2015). It is possible that Gray Triggerfish are determinate elsewhere in their range, which extends from Nova Scotia to Argentina in the western Atlantic (Hoese and Moore 1998). Building on the review in McBride et al. (2015) our findings support a conclusion that such mixed reproductive attributes may not be uncommon in fish and may be predicted to occur in lower latitudes in combination with energetically demanding accessory reproductive activities such as female parental care.

Batch Fecundity— Our findings on the reproductive strategy of Gray Triggerfish in the Gulf support using fecundity methodology based upon batch size and spawning frequency. Regardless, applying an indeterminate fecundity methodology is the cautionary approach when there is uncertainty about fecundity type (Gordo et al. 2008, Lowerre-Barbieri et al. 2011, Fitzhugh et al. 2012). However, Gray Triggerfish are rarely sampled in oocyte maturation (OM) or in the hydrated stage which is typically the basis for identifying a spawning batch. The difficulty of detecting OM in female Gray Triggerfish seems to be a common problem (Wilson et al. 1995, Hood and Johnson 1997, Moore 2001). This could be related to the lack of oocyte hydration during proteolysis, or reduced feeding prior to spawning leading to low susceptibility of females undergoing OM to hook and line capture (Wilson et al. 1995, Moore 2001, Simmons and Szedlmayer 2012). MacKichan and Szedlmayer (2007) report a mean (± SE) egg diameter of 620 ± 3 μm from nest excavations, which is close to mean ova size (602 ± 0.72 μm) from the one female we detected undergoing yolk coalescence and considered to be in spawning condition. In contrast, broadcast spawners with positively buoyant eggs, such as Red Snapper (*Lutjanus campechanus*), have egg diameters of ~820 μm (Rabalais et al. 1980). A lack of hydration would result in a much shorter period in which to collect imminent spawners, which in turn would yield a low incidence of actively spawning individuals. A similar difficulty occurs with sardines (*Sardina pilchardus*) and is attributed to a relatively long (>10 d) spawning interval (Ganias et al. 2004). Due to the infrequent capture of Gray Triggerfish undergoing OM, the spawning batch size separation method (Ganias et al. 2004) was used to calculate batch fecundity. The oocyte

size hiatus that develops as secondary oocytes advance provides the basis to define the spawning batch.

Other investigators of Gray Triggerfish fecundity have applied various criteria based on oocyte size or stage to define the spawning batch. Ingram (2001) reported 400 µm as the diameter that delimits the beginning of the spawning batch based upon the assumption this size corresponds to the onset of oocyte maturation. Using this criterion, mean oocyte density was estimated as 8,015 (±247) oocytes per gram of ovary (SEDAR9 2006 citing Ingram 2001). However, we found no evidence of oocyte maturation occurring for oocytes ≤590 µm oocyte in diameter. The only imminent spawning individual in our collections had a mean oocyte diameter of 602 µm (± 0.72) for oocytes undergoing OM. Given our finding that secondary oocyte development is group synchronous, this criterion would likely identify only a partial batch. Hood and Johnson (1997) indicated that the spawning batch could be identified as the standing stock of vitellogenic oocytes within an ovary, essentially the conclusion we arrived at. Their results for batch fecundity ranged from 0.2-1.2 million eggs with a density of 13,809 (±6,122) oocytes per gram of ovary. Our estimated oocyte density of 24,468 ±1,086 oocytes/g of ovary was much higher than the estimates of either Hood and Johnson (1997) or Ingram (2001). The difference between estimates calculated by us and Ingram (2001) may be readily accounted for by the difference in minimum oocyte size for defining a spawning batch (250 vs 400 µm). The difference between estimates calculated by us and Hood and Johnson (1997) is more difficult to account for, and we can only speculate that it was due to unaccounted factors such as enumeration of vitellogenic oocytes before a hiatus had formed in the oocyte size distribution or other effects based upon year, mean body size, or season etc. There are other fecundity estimates (Manooch and Raver 1984, Bernardes and Dias 2000), but details provided on methods and oocyte size or stage criteria for fecundity counts are not sufficient for a comparison. The range of our batch fecundity estimates (0.34 – 2.0 million eggs) was similar to counts of eggs made from 9 nests (0.42 - 1.4 million eggs) in Mackichan-Simmons (2008).

Spawning frequency—As found in many studies, spawning frequency can be difficult to assess due to low sample sizes and uneven sampling through the reproductive season (Stratoudakis et al. 2006, Ganias et al. 2011). Similar to the rarity of fish undergoing oocyte maturation, the

occurrence of females exhibiting POFs was comparatively low. This necessitated aggregating the data across years, which assumes no year effect, such that the proportion of females bearing POFs could be used to calculate spawning interval (the POF method = 7.6 d). In one year (2001), however, samples were evenly distributed over June and July to estimate spawning interval using the ISI method (11 d). This estimate depends on a few assumptions: 1) that POF degeneration offers a clock by which oocyte growth can be estimated and that maximum POF surface area we observed (12 μm^2) is representative of a new day-0 POF in Gray Triggerfish and 2) that the relationship regarding rate of POF degeneration to secondary oocyte growth is constant over the spawning season. We think the first assumption is reasonable based on the corresponding observation that cortical alveolar oocytes were the leading gamete stage and that provisioning of yolk for the subsequent batch had not yet begun. Also the relationship between POF degeneration and secondary oocyte growth was linear for our example from 2001. For the second assumption there is evidence from the literature that POF degeneration and secondary oocyte growth are both temperature dependent (Kjesbu et al. 1998, Ganias 2012). We also expect that water temperature and thus rates may change over a several months long spawning season. However, a simple assumption may be that any changes in rate of oocyte growth are proportional to changes in rate of POF degeneration. Further work is needed to test this assumption. For the purposes of this paper, the POF method and ISI method were needed to support each other due to the low sample size of individuals with POFs. A study of Yellow Margin Triggerfish (*Pseudobalistes flavimarginatus*) found that spawning grounds were vacated for 10 days between spawns on Australia's Great Barrier Reef (Gladstone 1994). This would be similar to the 11 d spawning interval we calculated for Gray Triggerfish. Earlier estimates of spawning interval for Gray Triggerfish varied greatly, from 15-37 d with in the U.S. South Atlantic (Moore 2001) to 3.7 d for Gulf Triggerfish (Ingram 2001).

In summary, female Gray Triggerfish in the Gulf exhibited mixed reproductive attributes apparently associated with the warm water environment and energetically demanding defense and parental care. Fecundity type in the Gulf should be considered indeterminate and the hiatus in size distribution of advancing secondary oocytes can be used to identify a spawning batch. Our estimated scope for number of batches produced during a spawning season is lower (8-11 d) than estimated elsewhere in the Gulf (c.f. Ingram 2001) but further research is needed. In

particular, age or size-dependency in batch number will be difficult to assess due to nesting behavior and parental care.

Further field collection efforts could improve fecundity estimates for Gray Triggerfish. Due to nesting and territorial behavior, SCUBA and spear fishing are necessary to readily identify and collect females in spawning condition. A survey could be designed to improve our knowledge of the timing and duration of spawning markers by targeting prespawning and postspawning females, in conjunction with visual observations of color phase and female size on nests, and measurements of environmental variables, perhaps most importantly, water temperature.

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Table 1. Number of samples obtained for each body index measure (Fulton's Condition Factor, Gonadosomatic Index, and Hepatosomatic Index) from various fisheries independent and fisheries dependent sources in the eastern Gulf of Mexico for the years 1999-2012. The gears are Hook and Line (includes bandit reel; HL), Trap (TR), and Spear (SP).

Source	Sampling Gear	Fulton's Condition Factor	Gonadosomatic Index	Hepatosomatic Index
Fisheries Independent	HL+TR	420	552	204
Fisheries Dependent	HL+SP	672	77	34
Total		1092	629	238

Table 2. Fecundity metrics of Gray Triggerfish and associated fork length information of females used for fecundity analysis.

	Fork Length (mm)	Somatic Relative Fecundity (oocytes/g OFBW)	Oocyte Density (oocytes/g ovary weight)	Batch Fecundity	Secondary Oocyte Diameter (µm)
Min	266	590	12,653	339,605	250 (limit)
Max	386	2,686	47,688	1,990,861	590
Mean (±SE)	311.3 (±3.6)	1,356.9 (±41)	24,468.82 (1085.64)	932,908.7 (±41458.6)	370 (±0.1)

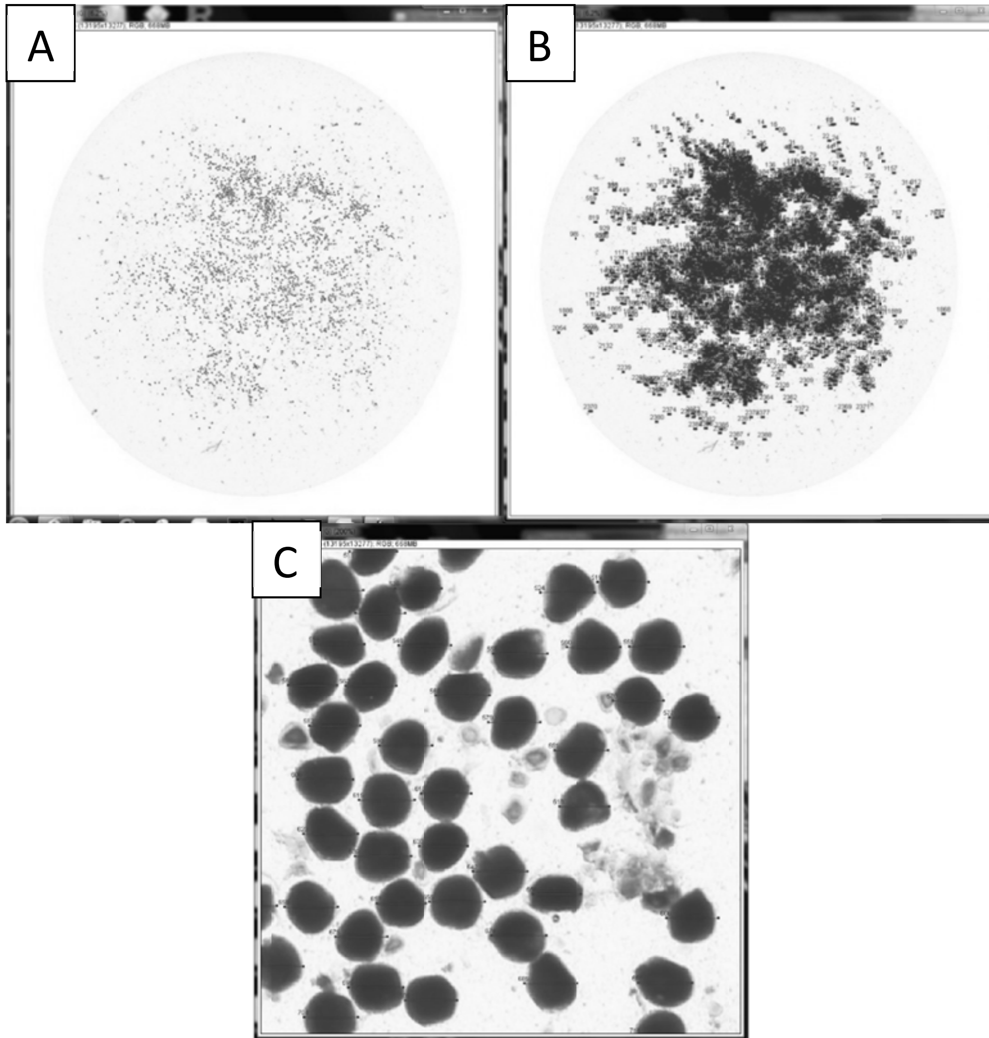

Figure 1. Whole mount ovary plates were scanned in a 150 mm Petri dish and screen captures are shown for A) lowest scan magnification, B) Object J delineation of secondary oocytes and C) highest scan magnification.

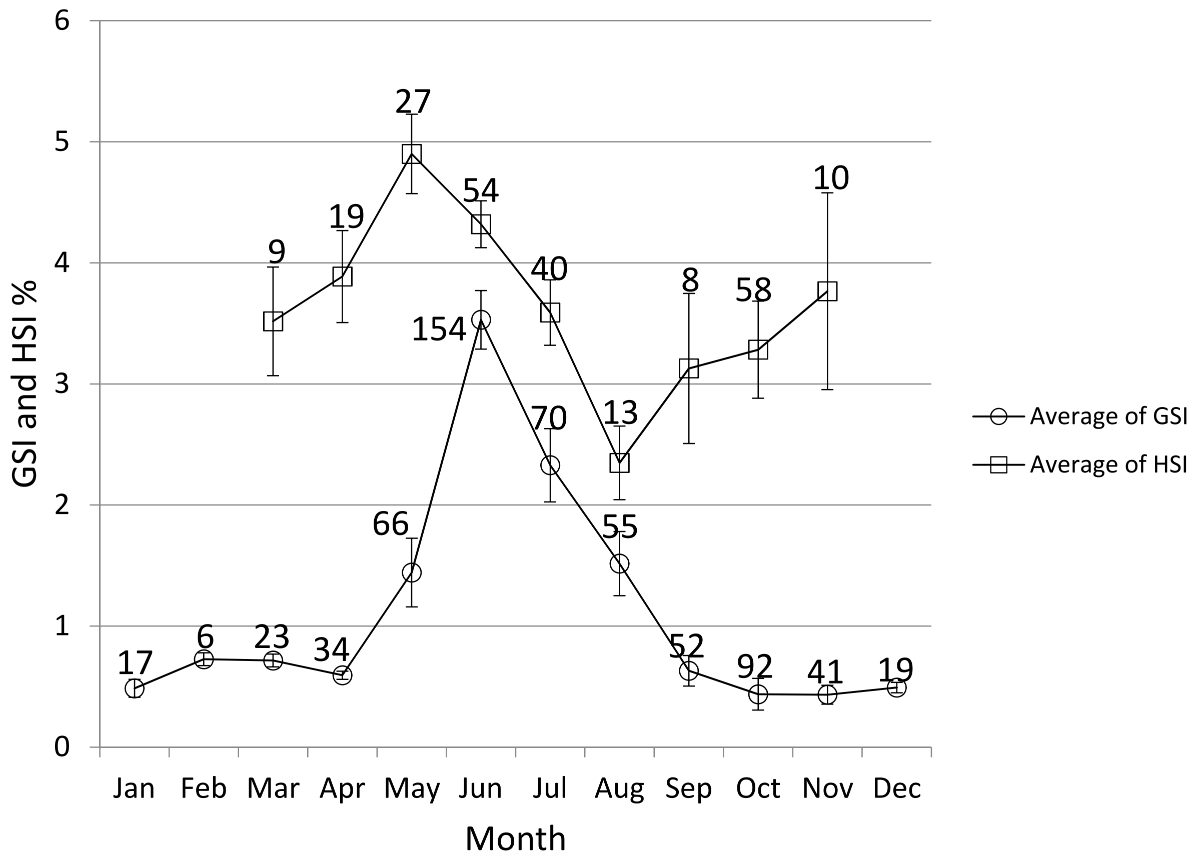

Figure 2. Female Gray Triggerfish gonadosomatic index (GSI) and hepatosomatic index (HSI) by month (mean ± one standard error) with n for each month.

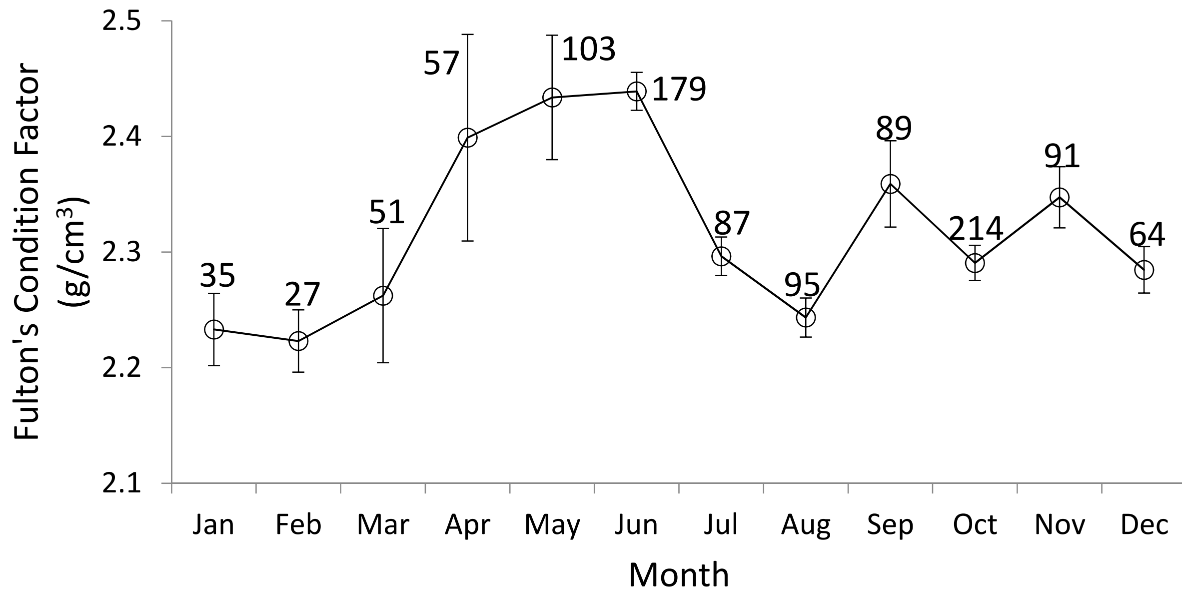

Figure 3. Female Gray Triggerfish Fulton's condition factor by month (mean ± one standard error) with n for each month.

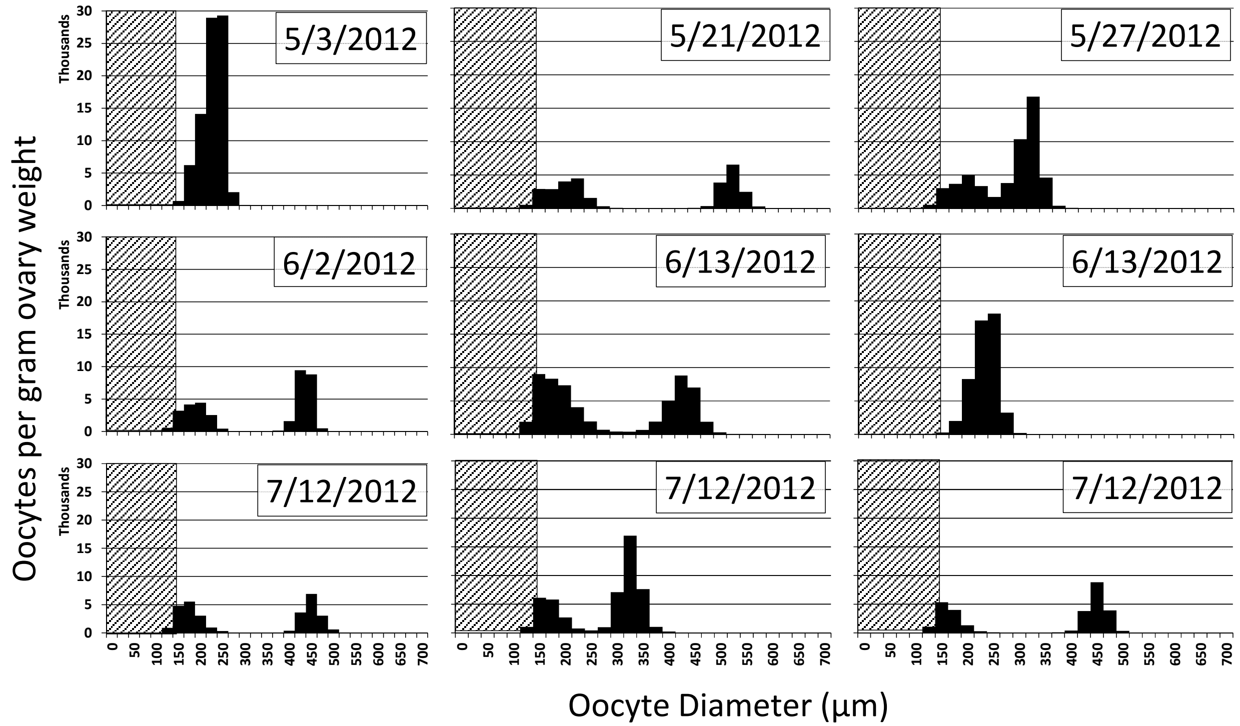

Figure 4. Oocyte size frequency (per g of ovary weight) by date for 9 individual Gray Triggerfish (3 randomly selected females per month). Dates of capture are displayed for each fish. Bins with gray shading correspond to sizes of primary growth oocytes.

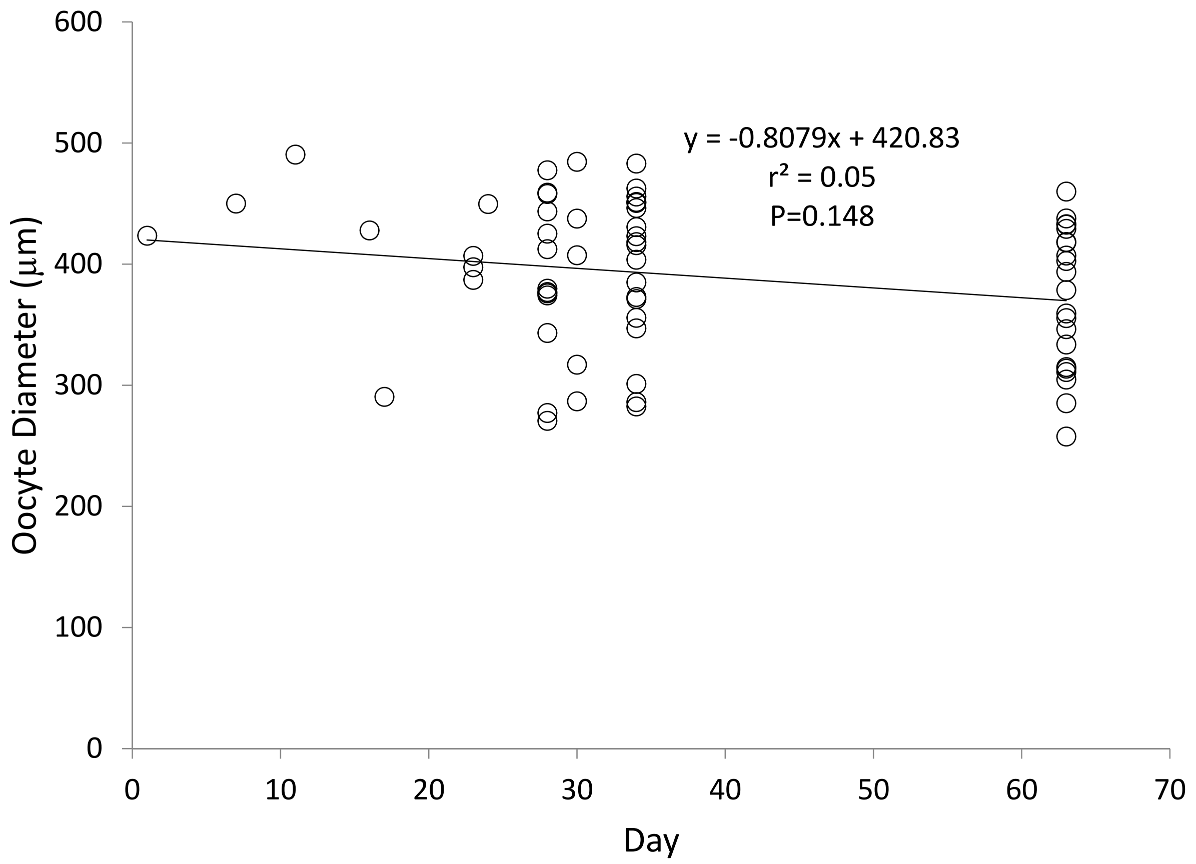

Figure 5. Gray Triggerfish mean oocyte diameter (µm) per individual female by day starting from the first day (11 May 2012) of collection within the 2012 spawning season.

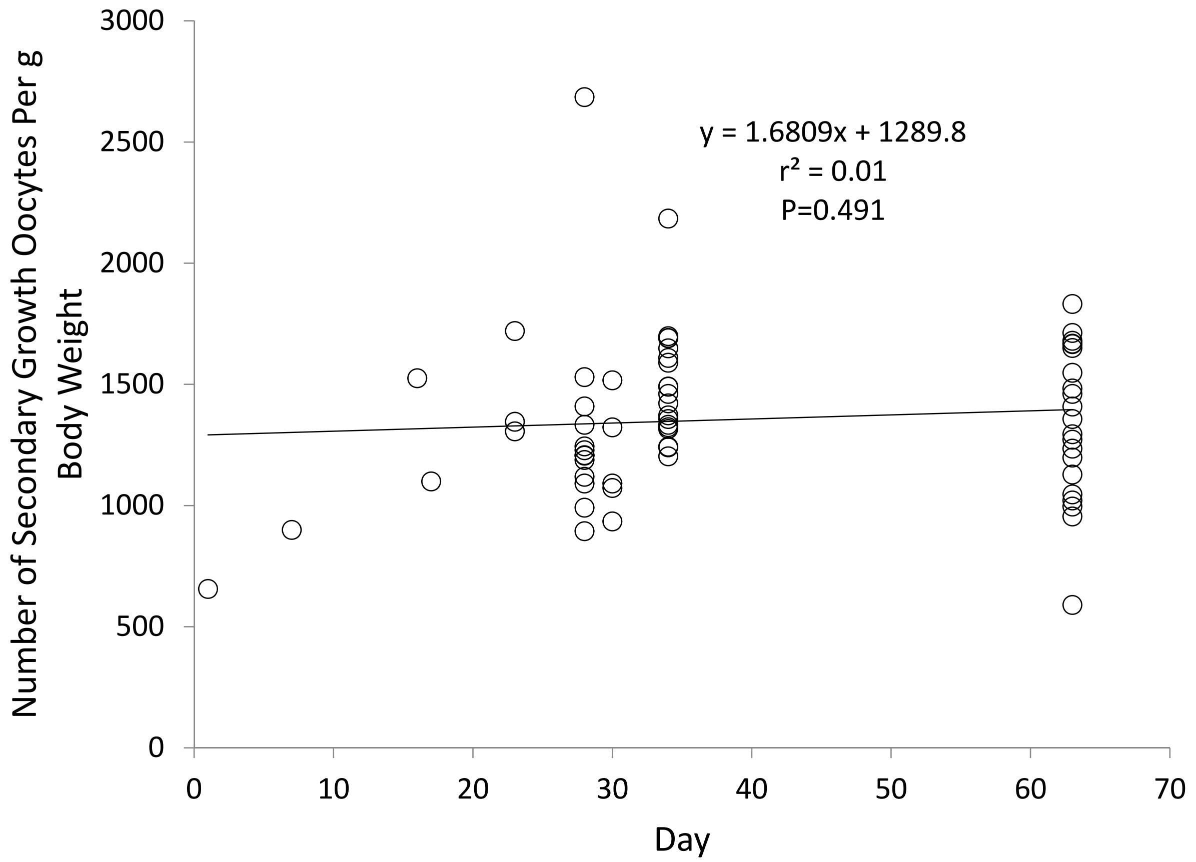

Figure 6. Abundance of Gray Triggerfish secondary growth oocytes by day starting from the first day (11 May 2012) of collection within the 2012 spawning season.

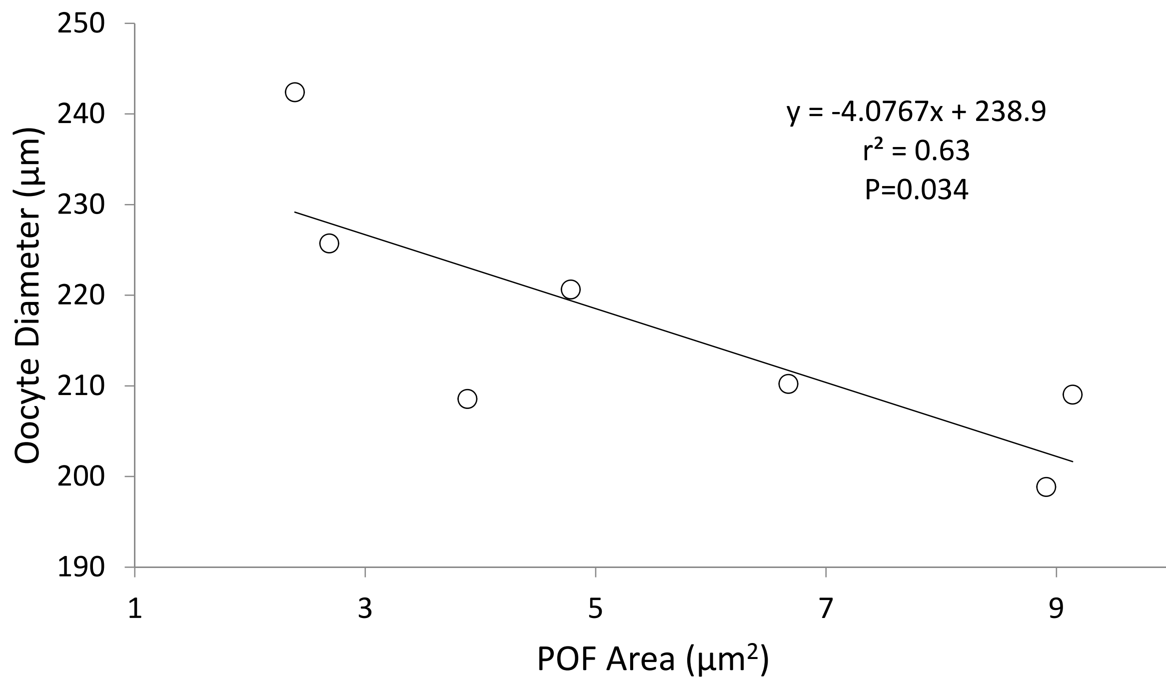

Figure 7. Gray Triggerfish mean oocyte diameter (µm) plotted against mean postovulatory follicle area (µm^2). Samples from seven females selected from 2011 (see text).

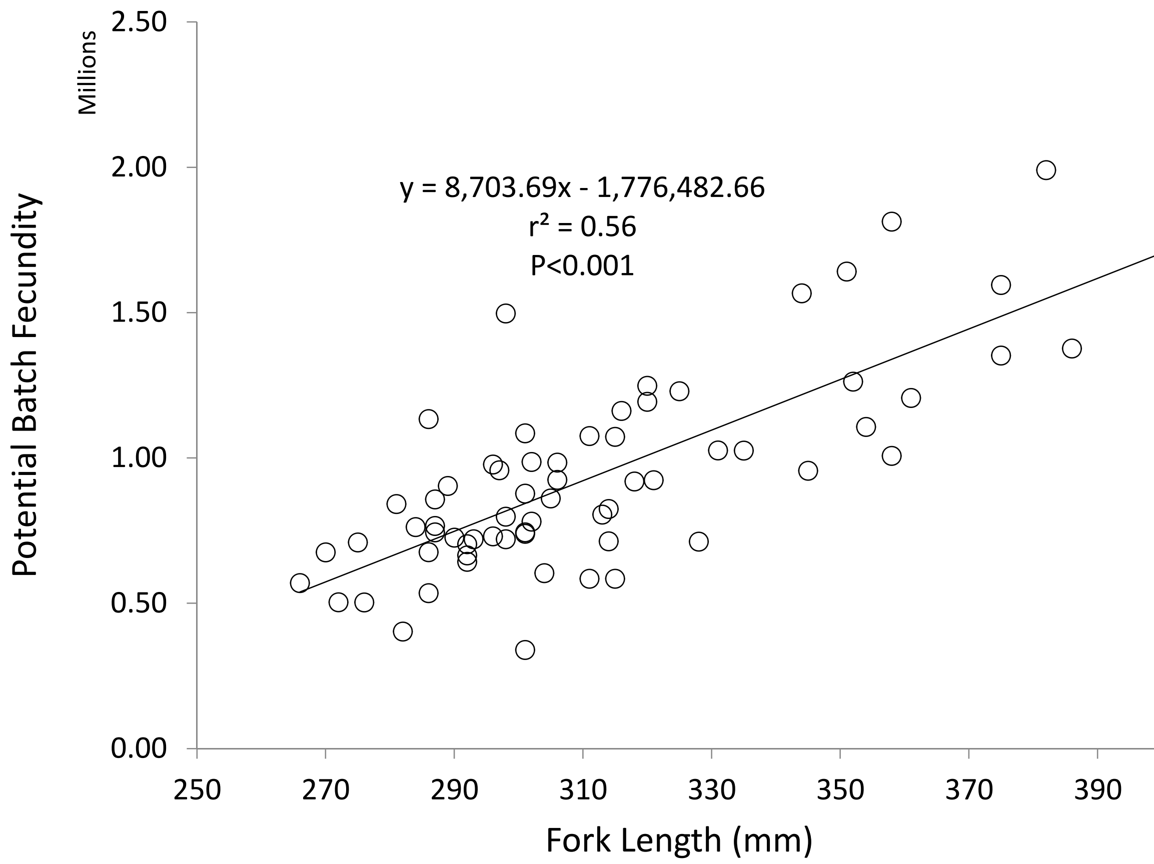

Figure 8. Gray Triggerfish potential batch fecundity at fork length (mm).