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## A Snapshot of the Age, Growth, and Reproductive Status of Gray Triggerfish (*Balistes Capriscus*, Gmelin 1789) on Three Artificial Reefs in the Northwest Gulf of Mexico

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A SNAPSHOT OF THE AGE, GROWTH, AND REPRODUCTIVE STATUS OF GRAY  
TRIGGERFISH (*BALISTES CAPRISCUS*, GMELIN 1789)  
ON THREE ARTIFICIAL REEFS IN THE  
NORTHWEST GULF OF MEXICO

A Thesis

by

ADAM M. LEE

Submitted to the Graduate College of  
The University of Texas Rio Grande Valley  
In partial fulfillment of the requirements for the degree of

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May 2019



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## ABSTRACT

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Age, growth, and reproductive status of gray triggerfish (*Balistes capriscus*) were identified from 2015-2016 on artificial reefs in the northwest Gulf of Mexico. Individuals ranged from 232-432 mm fork length with a mean of 319 mm. Individuals from age 0.2 to 5.2 yrs were observed with a weight to length relationship of  $Wg = 1.1 \times 10^{-4} \times FL^{2.7}$  ( $r^2 = 0.94$ ,  $n = 112$ ), where FL = fork length (mm) and Wg = weight (g). A von Bertalanffy growth equation of  $L_t = 326(1 - e^{-0.9(t+1.71)})$  was calculated irrespective of sex. Gonadosomatic index and histological characterization of reproductive tissue identified June-August as the peak spawning season. A female length to batch fecundity (BF) relationship of  $\text{Log BF} = 2.79 \times \text{Log}(FL^{0.81})$  ( $r^2 = 0.28$ ) was identified. Continued management of gray triggerfish on artificial reefs is necessary to increase the stock and eventually lead to robust and sustainable fisheries.





## DEDICATION

This enormous body of work is dedicated to the people who have guided, supported, and loved me through this project of passion. This achievement is a collage of those people reflected through a lens of my actions. This is for my father, Michael A. Lee, a man whose kindness, sacrifice, and stories of a universe beneath the sea flooded my imaginations since childhood. This is for my mother, Pamela P. Lee, her capacity for compassion and understanding are an unwavering force in times of turmoil and loss, joy, and elation. A friend, mentor, and inspiration who is genuine, open, and loving. She has taught me more than anyone about being my best self. This is for my partner, Elyssa M. L. Davis who has been ever an advocate and support for my progression, betterment, and passions, without who I could not have completed this amazing achievement. Through surprises, adventures, misadventures, strife and elation she has been the most important provider of perspective and understanding. This work is as much hers as it is my own. For these individuals, I am forever grateful.



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I must acknowledge the contribution of Dr. Carlos Cintra-Buenrostro, my thesis committee chair and mentor during this endeavor. Without his innumerable invaluable advice, guidance, and advocacy this project would have been impossible. His dedication, support, and insights provided the environment for this achievement. He is an esteemed and respected mentor who has my unyielding gratitude. In addition, the funding, support, advice, and comments of Dale Shively and Dr. Brooke Shipley of Texas Parks and Wildlife Artificial Reef Program must be recognized as essential and appreciated. My thanks go out to my committee members Dr. Richard Kline and Daniel Provenzano, whose expertise, pragmatism, and acumen have ensured my work is of the highest quality.

Without the assistance and input of many of the graduate school students, especially in the Ocean, Coastal, and Marine Sciences and Biology programs this project would not have been completed with efficiency or quality. My thanks go out to them, especially those whose time, effort, and ideas were instrumental in for collecting and processing individuals for this project.



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## CHAPTER I

### INTRODUCTION

#### **Background and Significance**

##### **Gray Triggerfish Biology and Ecology**

The gray triggerfish (*Balistes capriscus*, Gmelin, 1789) is a ubiquitous member of the northwest Gulf of Mexico reef community. Gray triggerfish are found throughout the tropical and temperate zone of the coastal Atlantic Ocean, the Mediterranean, and the Gulf of Mexico with bathymetric distribution of 100 m (Antoni et al., 2011; Simmons & Szedlmayer, 2011). In the Gulf of Mexico, gray triggerfish are most commonly found at depths of 12-42 m along reef and hard bottom habitats (Lyczkowski-Shultz et al., 2003). Juvenile gray triggerfish are recruited to reef and hard substrate sites by age of < 1 year with an observed maximum age of 15 years (Southeast Data Assessment and Review [SEDAR], 2015). Most landed individuals are among ages 2-6 with individuals older than 10 years rare among surveys (SEDAR, 2015). Gray triggerfishes are infamously difficult to age, with small (< 2 mm) and inconspicuous otoliths characterized by irregular accretion patterns (Allman et al., 2015). First dorsal spines have been validated as a method to reliably age gray triggerfish (Allman et al., op. cit.). These reef members have a unique body plan which exhibits dorso-lateral flattening, a balistiform locomotion (Sfakiotakis et al., 1999) and tough leathery skins. The caudal fins of gray triggerfish exhibit long decorative extensions and therefore body length is preferentially measured from mouth to tail fork (fork length = FL). Inquisitive, curious, and notoriously aggressive (Lieske &

Myers, 1999), gray triggerfish exhibit site specificity (observed exclusively on a narrow range of habitats) and site fidelity (individuals do not migrate among and between habitat sites) (Lingo & Szedlmayer, 2006).

Spawning in the Gulf of Mexico has been observed from April to September with greatest intensity between May and August (Ingram, 2001). Gray triggerfish reach reproductive age at age 1 year for males and age 1.5 years for females (Simmons, 2001). Batch fecundity has been observed to be strongly correlated with FL. An intermediate fecundity reproductive pattern is identified in female gray triggerfish with an 86-day reproductive period during which spawning can occur every 8-11 days (Burton et al., 2014). Gray triggerfish exhibit an atypical spawning behavior, forming harem groups (consisting of up to 5 females) and provide parental care of eggs for 24-48 h after spawning (Lieske & Myers, 1999; Ingram, 2001; Simmons & Szedlmayer, 2011).

The observed sex ratio identified in the SEDAR report which represents a Gulf of Mexico wide population survey is skewed towards females during histological (1:1.06 [56%]) and macroscopic (1:1.16 [64%]) observations in the Gulf of Mexico. However, the current federal and state stock assessment maintains a sex ratio set at 1:1 for management purposes (Fitzhugh et al., 2015; Gulf State Marine Fishery Council, 2015).

Gray triggerfish populations have been shown to be genetically homogeneous within the Gulf of Mexico (Antoni et al., 2011), with biomass distribution biased toward the eastern Gulf of Mexico as there is an abundance of the gray triggerfish's preferred natural hard bottom habitat (Mueller, 2012; SEDAR, 2015).

## **History of Gray Triggerfish Management in Federal and State Waters**

The Magnuson-Stevens Fishery Conservation and Management Act of 1976 attempted to restore United States (US) fisheries in marine ecosystems to healthy, sustainable levels through implementation of major fisheries management plans (National Oceanographic and Atmospheric Administration [NOAA], 2015). The Reef Fish Management plan of 1984 was the first attempted legislation to identify and mitigate factors which depleted stocks of reef associated fishes in US waters. Assessments of the gray triggerfish population within the Gulf of Mexico were performed in 2001, 2006, 2011, and 2015 by the Gulf of Mexico Fisheries Council to enact management policy intended to revitalize an overfished species which had seen steep declines in biomass from the 1950s onward (SEDAR, 2015). However, these measures did not facilitate recovery, and stocks continued to diminish (SEDAR, *op. cit.*).

As a cooperative measure during the same period, Gulf of Mexico states' regulatory agencies enacted standardized management policies in 1992, 1994, 1996, 2003, and 2005 which established bathymetric inshore boundaries and standardized minimum collection limits (size and number), and outlawed specific high yield gear types (SEDAR, 2015). These measures did not lead to a gray triggerfish stock rebound during this period and more strict regulations were enacted to overcome the losses (SEDAR, *op. cit.*). However, stocks continued to decline and, to further protect the population both state and federal gray triggerfish fisheries were closed in most Gulf of Mexico states and a moratorium was imposed in federal waters as of December 2017.

## **Local Gray Triggerfish Management**

Texas Parks and Wildlife Department (TPWD) is responsible for gray triggerfish fishery management in state marine waters, defined as the area from shore to 16.7 km (9 NM) (TPWD,

2017). Texas currently adheres to federally mandated bag (20 individuals daily) and size limits (406 mm or 16 in FL) during the year-round recreational fishing season, a break from the permanently closed federal fishery and restricted fisheries of the other Gulf of Mexico states (Alabama Department of Conservation and Natural Resources, 2017; Florida Fish and Wildlife Conservation Commission, 2017; Louisiana Department of Wildlife and Fisheries, 2017; Mississippi Department of Marine Resources, 2017; TPWD, op. cit.).

Florida (except for a 10-day recreational season) and Alabama are in an indefinite fishing moratorium as of December 2017. Louisiana regulations mandate a lower bag limit and the fishery is closed during spawning season. Other than Texas, only Mississippi maintains a yearlong open season, with federal size limits and bag limits observed (Alabama Department of Conservation and Natural Resources, 2017; Florida Fish and Wildlife Conservation Commission, 2017; Louisiana Department of Wildlife and Fisheries, 2017; Mississippi Department of Marine Resources, 2017; TPWD, 2017).

### **Artificial Reefs in the Northwest Gulf of Mexico**

Natural hard substrates in the Gulf of Mexico are rare (~ 8%): bottom substrate consists mostly of flat sand, mud, and gravel (Mueller, 2012). Only 1/100<sup>th</sup> of a percent of an available 349,100 km<sup>2</sup> of essential fish habitat in the Gulf of Mexico is artificial structures (Mueller, op. cit.) (Figure 1). Except for few poorly documented remnant natural reefs, few extensive natural hard bottoms are available within Texas state waters (TPWD, 1990). Artificial reefs are “islands of opportunity,” providing substrate for habitat limited sessile invertebrates and algae (collectively known as biofouling), which in turn support motile invertebrate and fish species.

This trophic support helps develop dynamic environments that increase biomass at the fouling sites.

These localized biomasses enhance recreational fisheries, diving, and tourism (Brock, 1994). As a result, the Texas legislature enacted the Artificial Reef Act in 1989, which directed TPWD to “develop, maintain, monitor, enhance and promote artificial reef potential in state waters and federal waters adjacent to Texas” (TPWD, 1990). Artificial reef monitoring and evaluation program in south Texas began with the deployment of the Texas Clipper Artificial Reef in 2007 (Hicks & Cintra-Buenrostro, 2008). This site provided opportunities to address questions regarding the productivity, general ecology, and use of artificial reefs by ecologically and economically valuable reef species, fishermen, and recreational spectators. Artificial reef benefits were clearly demonstrated in studies of juvenile fish recruitment, fish communities, and fish habitat use on artificial structures in the northwest Gulf of Mexico during the past 5 years (Froehlich & Kline, 2015; Arney et al., 2017).

### **Study Objectives**

While it is established that artificial reefs provide a haven for larval and pelagic organisms and increase the number of juvenile fishes, the age, growth, and reproductive status of gray triggerfish on these structures have not been thoroughly investigated. Artificial reef sites sampled in this study are the southernmost hard-bottom structures within US waters in northwestern Gulf of Mexico and can provide a basis for future study in this region (Bortone et al., 1994; Wilson et al.; 1995 Carr & Hixon, 1997; Bortone, 1998). Gray triggerfish age, growth, and reproductive patterns in the northwest Gulf of Mexico are important to investigate because of gray triggerfish’ vulnerability to fishing pressures. Observed site fidelity and site specificity in



combination with a scarcity of natural and artificial habitat in the northwest Gulf of Mexico indicated that an absence of refuge from fishing deleteriously impact gray triggerfish populations compared to other, supportive habitats (Addis et al., 2007; MacKichan & Szedlmayer, 2007; Lombardi et al., 2015). Artificial structures that contribute to rebuilding of gray triggerfish stocks are particularly important to investigate because of prior failures in stock management.

Gray triggerfish on artificial reefs in the northwest Gulf of Mexico are hypothesized to exhibit: 1) a length and weight range and relationship similar to other populations of gray triggerfish elsewhere in the Gulf of Mexico; 2) size divergence between inshore and offshore sites; 3) age range and composition similar to other populations in the Gulf of Mexico; 4) size-at-age comparable to other populations found in the Gulf of Mexico; 5) a 1:1 sex ratio; 6) a peak spawning season similar to other populations found on other artificial reefs in the Gulf of Mexico; 7) shared maturation characteristics with other populations found in the Gulf of Mexico; and 8) an increase in batch fecundity (and subsequent increase of annual fecundity) with age, weight, and FL.

The identification of a single genetic population (Antoni & Sallient, 2011) and its association with a specific habitat type across the Gulf of Mexico suggested that south Texas gray triggerfish would exhibit length, weight, age, growth pattern, and reproductive characteristics and traits similar to other reef fish populations in the Gulf of Mexico. For example, a recent study on red snapper (*Lutjanus campechanus*, Poey 1860) at artificial reefs sampled along the South Texas coast revealed a significant increase in length and weight of individuals on reef sites further from shore (Alexander, 2015). This kind of variation in a species that occupies a similar trophic dynamic, habitat, and global distribution suggests similar patterns might be found for gray triggerfish (Thera & Rumbold, 2014). Gray triggerfish form

reproductive harems of one male and up to five females, and while this behavior suggests a population dominated by females, assessments of sex ratio Gulf-wide identify sex ratios dominated by either males (Wilson et al., 1995; Hood & Johnson, 1997) or females (Moore, 2001; Fitzhugh et al., 2015). Additionally, it is well established that fishes generally increase their reproductive capability with an increase in size and age (Hixon et al., 2004), and other studies of gray triggerfish in the Gulf of Mexico have identified increases in size in association with reproductive output (Ingram, 2001; Fitzhugh et al., op. cit.). By identifying the fork length at which the majority of gray triggerfish are mature provides a passive monitoring method for local populations and allows for the identification of a relationship between maturation and age as a relationship between age and fork length is well established.

## CHAPTER II

### METHODS

#### **Field Data Collection**

##### **Study Location**

Three artificial reef sites, two in state waters (< 15 km from shore) PS-1169L (PI) and PS-1047 (PM) and one in federal waters (> 15 km from shore) PS-1070 (LB), were sampled seasonally (Winter, Spring, Summer, Fall) from January 1<sup>st</sup>, 2016 until January 1<sup>st</sup>, 2017. The southernmost artificial reef site (PI), 7.96 km from shore, is composed of two, three-pile jackets (reefed oil rigs), 53 reef balls, and three vessels ranging from 18-45 m in length at an average depth of 21.8 m. All reef substrate for PI was deployed in July of 2011. The northern most reef site (PM), 10.37 km from shore, consisting of over 4,800 concrete culverts distributed in dense and loose aggregates was deployed in 2011, in addition to a 30 m tugboat deployed in 2009 are located at an average depth of 19.7 m. LB was the only reef located in federal waters (20.19 km from shore) and consists of three ships and 15 assorted four-pile jackets at a depth of 30.9 m. Detailed descriptions and images of the sites can be found in Bollinger & Kline (2017).

The initial harvesting protocol consisted of a standardized vertical long fishing line adapted and modified from SEMAP (2012). However, collecting gray triggerfish with a hook-and-line system designed to target reef fishes that swallow prey such as red snapper and red grouper (*Epinephelus morio*, Valenciennes, 1828) was not productive to land grey triggerfish because of their small mouth gape (Nelson & Bortone, 1996; Burns & Froeschke, 2012).

Consequently, in this study, the collection of individuals was restricted to spearfishing. While this method presented inherent biases, such as an inability to collect small individuals and fish conditioned for avoidance behavior (Dickens et al., 2011), it is not an uncommon collection method for this species (Ingram, 2001; Simmons, 2001; MacKichan & Szedlmayer, 2007) and has been considered less biased than other collection methods (Randall, 1967). All collected individuals were stored on ice and processed within 48 h of capture.

## **Laboratory Data Collection**

### **Morphometrics**

Morphometric measures from dissected specimens included: FL  $\pm$  0.1 mm, weight  $\pm$  0.1 g, and gonad weight  $\pm$  0.01 g. Weight as a function of FL was generated using a generalized linear regression. Sex was determined macroscopically, and deviations from a 1:1 sex ratio determined with a Chi-Square test (Sokal & Rohlf, 2012).

### **Age**

First dorsal spines provide a validated age structure as an alternative to otoliths, which are inconspicuous and difficult to extract in gray triggerfish (Allman et al., 2015). The first dorsal spines were extracted and immediately placed at  $-40^{\circ}\text{C}$  to avoid vascularized tissue damage, as tissue damage can compromise the quality of the spines during later analysis. Dorsal spines were cleaned of extra tissue and dried for 24 h at  $60^{\circ}\text{C}$ . The spine was sectioned above the peduncle and mounted on glass slides with clear epoxy. Affixed spines were cross-sectioned at 0.5 mm thickness with an MTI Corp 150 low-speed diamond saw. A multiphase microscope was used to independently identify the number of annulations and assign a margin code based upon

methods described by Allman et al. (op. cit.). The accumulated variance (ACV) between readers was below 5% (ACV = 2.97) and no spines were omitted from the analysis. Age was determined within  $\pm 0.1$  years of age employing the annulation-count method described above by Lombardi et al. (2015).

## **Growth**

Age and the FL of individuals were employed to generate three-parameter von Bertalanffy length-at-age growth curves. The von Bertalanffy growth curve has a generalized format of:

$$L_t = L_\infty * 1 - e^{-k(t-t_0)}$$

For this study  $L_t$  = fork length at age  $t$ ,  $L_\infty$  = asymptotic length where growth rate = 0,  $k$  = growth rate,  $t$  = age (years  $\pm 0.1$ ), and  $t_0$  = theoretical age at which FL = 0. To compare the populations' length-at-age von Bertalanffy equations used in other gray triggerfish studies, FL-at-age was calculated to the nearest FL ( $\pm 1$  mm) at 1.0-year intervals (starting at zero) for the biological age range presented in this study's sampled population and the observed lifespan of the species. The calculated lengths were then compared among studies with a one-way ANOVA (Johnson & Saloman, 1984; Escorriola, 1991; Moore, 2001; Ingram, 2001; Bernardes, 2002; Burton et al., 2014; Kacem et al., 2015b). If significant differences were observed a Tukey HSD test was used post-hoc to identify homogeneous subsets.

## Reproductive Characteristics

The gonadosomatic index (GSI) of individuals was calculated to determine peak spawning season, month of peak spawn, and batch fecundity with modified methods from Branco et al. (2013) where:

$$\text{GSI} = \text{Gonad Weight/Weight} * 100$$

A GSI of 1 or greater indicates that individuals are capable of spawning (Kjesbu, 2009). The GSI of female gray triggerfish were aggregated and averaged among months captured, with average GSI used to determine both peak spawning season and a month of peak spawn. With peak span season characterized as the months where average female GSI >1 and month(s) of peak spawn characterized as the month where the greatest GSI monthly average was observed.

A histological characterization of gonadal tissue was required to determine batch fecundity, age at maturity, and FL at maturity. Gonad tissue was dissected, trimmed of excess fat, and immediately immersed in Prefer™ (glyoxal fixative) for at least 14 but no more than 30 days. After fixation, a random lobe and quartile of the gonad was selected for histological sampling. Each gonad lobe (right and left) and each lobe region (top, middle, lower, bottom) was assigned a value. A random number table was generated within the range of assigned values and used to select a lobe and region at random. After random selection, a 4-6 mm thick section was excised and patted dry of excess fluids and a KD-TS3D tissue cassette processing system impregnated tissue with paraffin wax. Tissue samples were embedded in paraffin blocks and thin-sectioned (5-10 µm) using a Richter-Jung 2030 rotary microtome. Tissue sections were mounted on a glass slide and stained with Gill's 2 hematoxylin and EOSIN-Y using a Tissue-Tek II slide staining kit.

Reproductive status of gonadal tissue was determined, following methods adapted from Kacem et al. (2015a), by two independent readers with a multiphase microscope. No gonad was excluded from the analyses. Gametogenic and accessory tissue reproductive stage characterization was adapted and modified from Brown-Peterson et al. (2011) and Kacem et al. (op. cit.) (Table 1). Maturity (mature or immature) was assigned to males or females who exhibited: spawning capable, actively spawning, and regressing, reproductive characterization.

Batch fecundity ( $1 \times 10^6$  oocytes) was calculated by selecting females histologically identified as either spawning capable or actively spawning, with a GSI above 1. These individuals ( $n = 12$ ) had ovarian tissue processed and mature oocytes counted as done by Hunter & Macewicz (1985).

To compare the populations' batch fecundity among studies of gray triggerfish from the Gulf of Mexico, batch fecundity as a function of FL was calculated at FL ( $\pm 1$  mm) at 25 mm intervals (starting at zero) for the FL ranges presented in this study's females and those ranges observed for the surveyed Gulf of Mexico population when appropriate. The comparison of batch fecundity between studies (Ingram, 2001 only) was also repeated with batch fecundity as a function of weight. Batch fecundity was compared among studies using a one-way ANOVA (Ingram, 2001; Lang & Fitzhugh, 2015). If significant differences were observed a Tukey HSD test was used post-hoc to identify homogeneous subsets.

Fork length at maturity and age at maturity were calculated for females only using methods adapted from Kacem et al. (2015a) and compared among studies from the natural range of gray triggerfish.

## Data Analyses

Statistical analyses were performed in SPSS V23 (IBM, 2013), with  $\alpha = 0.05$  unless otherwise stated. Age and morphometric (FL and weight) parameters were subject to tests of normality by the Kolmogorov-Smirnov test and visual Q-Q plots. Homoscedasticity was verified for age and morphometric parameters with Levene's test (Sokal & Rohlf, 2012). Oocyte count (the baseline value for batch fecundity calculations) was determined normal and homoscedastic by the Shapiro-Wilks and Levene's tests, respectively (Sokal & Rohlf, op. cit.).

A generalized linear model was used to generate weight as a function of FL to determine if FL was a significant predictor of weight. A generalized linear model was also used to generate batch fecundity as a function of FL or weight to determine if morphometric characters were significant predictors of batch fecundity. Additionally, the same model was used to determine if FL and or weight was a significant predictor of age.

A nested analysis of variance (NANOVA) was used to compare morphometric and reproductive dependent variables among location (inshore vs. offshore), with season of collection (Winter, Spring, Summer, and Fall), and sex (male or female) subsequently nested (Sokal & Rohlf, 2012). This approach was necessary because the further from shore individuals were expected to be larger and older.

The von Bertalanffy age and growth curves were calculated utilizing non-linear regression functions with methods adapted from Ahmad et al. (2015). All assumptions for the non-linear regression analysis were passed prior to testing. Calculated FL-at-age was compared with observed FL using a one-way ANOVA in order to identify the accuracy of the growth curve fit. Additionally, a one-way ANOVA was used to compare FL-at-age and batch fecundity of populations sampled among studies.



Fork length at maturity and weight at maturity were calculated using a binary logistic regression analysis adapted from Kacem et al. (2015b). Maturity, FL, and weight did not violate assumptions testing prior to binary logistic regression.

Sexual dimorphism has been reported in gray triggerfish throughout their natural range (Ingram, 2001; Bernardes, 2002; Burton et al., 2014; Allman et al., 2017). Subsequently, estimates of life or reproductive history are represented as: females only, males only, or a combination of both sexes (total/all) when appropriate.

## CHAPTER III

### RESULTS

#### **Morphometrics**

In total 112 gray triggerfish were collected from three artificial reef sites in the northwest Gulf of Mexico. The FL of gray triggerfish ranged between 232-432 mm, with a mean FL of  $315 \pm 39$  mm, males exhibited a smaller FL range (244-405 mm) than females (232-432 mm). Male and female gray triggerfish's mean FL was  $329 \pm 34$  mm and  $302 \pm 39$  mm, respectively. Observed mean  $\pm$  STD FL and FL ranges (total, male and female) for sampled sites are detailed in Table 2. The weight for gray triggerfish ranged between 0.302 g - 1.1564 kg with a mean weight of  $771.7 \pm 266$  g. Mean  $\pm$  STD weight (male:  $866.2 \pm 254$  g; female:  $689.8 \pm 250.2$  g) and range of weights (males: 349-1501.8 g; female: 302.9 g - 1.5648 kg) were observed with sampled site-specific values presented in Table 3.

Significant differences in FL were observed between males and females ( $F_{(6,111)} = 2.864$ ,  $p = 0.013$ ), with no significant differences observed owing to location, site, or season sampled. Significant differences were also observed in weight between males and females ( $F_{(6,111)} = 3.435$ ,  $p = 0.004$ ) with no significant differences observed owing to other factors (location, site and season). A similar pattern was identified in gonad weight with significant differences only present between sexes ( $F_{(6,111)} = 6.66$ ,  $p = 0.001$ ) with no differences in gonad weight associated with geography or season. Observed differences in gonad weight were expected as ovaries were (with rare exception) always heavier than testes in the gray triggerfish sampled. Site specific

gonad weights are also presented in Table 3. While no significant differences were attributed to factors other than sex; smaller fish were observed at PI, intermediate sized at PM, and the largest from LB.

### Age

The observed mean  $\pm$  STD ( $2.5 \pm 1$ ) and range (0.7-5.2) of age in the collected gray triggerfish was generally ( $\pm 0.1$ ) conserved between sexes, with site specific age composition presented in Table 4. No significant differences in the age of gray triggerfish were observed or attributable to differences among variables (location, site, collection season, or sex). Neither observed FL, nor weight was found to be significant predictors of age.

### Growth

Fork length was found to be a statistically significant predictor of weight in male ( $r^2 = 0.94$ ,  $p = 0.001$ ), female ( $r^2 = 0.93$ ,  $p = 0.001$ ) (Figure 2) and combined gray triggerfish ( $r^2 = 0.94$ ,  $p = 0.001$ ) (Figure 3) with relationships detailed in Table 5.

The von Bertalanffy FL-at-age three parameter growth curve calculated for females ( $FL = 313 \times 1 - e^{-0.9(t+1.624)}$ ), males ( $FL = 341 \times 1 - e^{-0.9(t+1.606)}$ ) and combined ( $FL = 326 \times 1 - e^{-0.9*(t+1.706)}$ ) (mm) (Figure 4). Calculated FL-at-age were not statistically different among the combined ( $F_{(1, 222)} = 0.06$ ,  $p = 0.81$ ), male ( $F_{(1,112)} = 0.01$ ,  $p = 0.92$ ), and female ( $F_{(1,112)} = 7.26 \times 10^{-5}$ ,  $p = 0.99$ ). Additionally, no observed significant difference ( $F_{(13,82)} = 1.509$ ,  $p = 0.11$ ) could be identified among calculated FL(s)-at-age when compared to six previous studies of gray triggerfish growth (Johnson & Saloman, 1984; Escorriola, 1991; Ingram, 2001; Moore, 2001; Bernardes, 2002;

Burton et al., 2014; Kacem et al., 2015b) throughout the natural range of the gray triggerfish (Figure 5) with the FL-at-age equations compared among studies detailed in Table 6.

## **Reproductive Characteristics**

### **Sex Ratio**

The overall sex ratio for this study was 1:1 male to female. However, sex ratio differed at the two inshore sites, but not at the offshore site. The site PI ( $\chi^2 = 5.0$ ,  $p = 0.025$ ) was dominated by females and the PM site ( $\chi^2 = 6.081$ ,  $p = 0.014$ ) was dominated by males.

### **Reproductive Status**

All ovarian and testicular reproductive stages were observed in female (Figure 6) and male (Figure 7) gray triggerfish, respectively. Female gray triggerfish sampled in July had the greatest average GSI at 8.4. Gray triggerfish caught in June, July, and August had a mean GSI  $\geq 3.3$ , greater than the other months sampled (Figure 8). From June to August 91.7% of ovaries sampled were identified as developing, spawn capable, actively spawning, or regressing. This indicated a peak spawning season observed from June to August, with a peak spawning month in July (Figure 8). While nesting behavior was observed at all collection sites (pers. obs.), histological assessments identified hydrated oocytes from a female collected at PM, which was the first confirmation of the use of artificial reefs by gray triggerfish throughout ontogeny in the northwest Gulf of Mexico region.

## **Maturity**

Proportional FL and weight at maturity were calculated for gray triggerfish females only. Females exhibited 50% maturity ( $FL_{50}$ ) at 230 mm FL (Figure 9a) and 50% maturity ( $Wg_{50}$ ) at a weight of 325 g (Figure 9b).

## **Reproductive Output**

Batch fecundity as a function of FL or weight were generated to compare results among studies of gray triggerfish populations in the Gulf of Mexico (Figure 10) with observed batch fecundity, batch fecundity ranges for females above  $FL_{50}$  and  $Wg_{50}$ , and summary statistics presented in Table 7. Log transformed batch fecundity as a function of log transformed weight was found to display the best fit ( $\text{Log BF} = 3.994 \times \text{Log}(Wg^{0.37})$ ,  $r^2 = 0.40$ ) (Figure 10) with linear and power relationships (Figure 11) described in Table 8. Oocyte distribution was not observed to differ significantly with regard to lobe (right or left) or lobe region (top, middle, lower, bottom). Age was a poor predictor of batch fecundity and was excluded (data not shown).

Females used to calculate batch fecundity as a function of FL were similar to sizes examined by Ingram (2001) and Lang & Fitzhugh (2015) (Figure 12). Batch fecundity, as a function of FL, estimates were statistically different among studies ( $F_{(3,99)} = 5.360$ ,  $p < 0.05$ ). In addition, batch fecundity as a function of weight was significantly different among studies ( $F_{(2,99)} = 4.378$ ,  $p < 0.05$ ).

## CHAPTER IV

### DISCUSSION & IMPLICATIONS

#### **Morphometrics**

##### **Length and Weight**

Longer, heavier, and older fish were expected to be observed on artificial reefs further from shore. This phenomenon was observed by Alexander (2015), in *L. campechanus* which share a similar place within the trophic dynamics on artificial reefs (Thera & Rumbold, 2014). While no significant difference in FL was observed among sites in this study, on average gray triggerfish increased in FL and weight as sites increased their distance from shore. Whether this phenomenon has been observed in populations of gray triggerfish on artificial or natural habitats elsewhere in the Gulf of Mexico is unknown.

Studies in the Gulf of Mexico and off the coast of Brazil have identified sexual dimorphism and differences in growth between males and females (Ingram, 2001; Bernardes, 2002; Burton et al., 2014; Allman et al., 2017) supporting differences in FL and weight between male and female gray triggerfish observed at artificial reefs in the northwest Gulf of Mexico.

##### **Sex Ratio**

Sex ratios varied at each sampling site: gray triggerfish sexed at the offshore site (LB) were found to adhere to a 1:1 sex ratio. Although only 19 females to 11 males were counted, it is

tempting to speculate that this sex ratio weighted towards females would be retained if more fish were observed at that site.

Macroscopic and histological assessments of gray triggerfish from previous studies support these findings with populations observed having a female skew as detailed by SEDAR (2015). However, whether a 1:1 sex ratio accurately reflects the population of this region remains unclear as findings from this study and others (Fitzhugh et al., 2015; Gulf State Marine Fishery Council, 2015) have observed populations with a close to 2:1 female bias at two of three sampled sites. Whether environmental or biological factors influenced sex ratio at sampled sites has not been determined; previous investigations of habitat use (Addis et al., 2007; Herbig & Szedlmayer, 2016; McKinzie & Szedlmayer, 2017) and spawning behavior (Ingram, 2001; Simmons, 2001) may provide further insights into the observed imbalance.

Gray triggerfish were observed to increase their average space utilization on artificial reefs in months before and after the peak spawning season (from June to August) in the Gulf of Mexico (Lang & Fitzhugh, 2015). This activity increase before the spawning is likely related to acquisition of both optimal nesting grounds and mates with a return to residential reefs accounting for the post-peak spawning season increase.

Males form harems of up to five females and aggressively protect their nesting sites and females (Lieske & Myers, 1999; Simmons & Szedlmayer, 2011). Dominant males can drive submissive males from coveted breeding females and nesting sites. The displacement of submissive males increases the search area for females which have not been recruited into harems. Additionally, female aggregation around optimal nesting habitats might contribute to sex-ratio imbalances. Whether a combination of factors aggregates males onto suboptimal habitat or aggregates females at optimal habitat is speculative at this time.

## Age

Due to the lack of significant differences observed and challenges associated with identification of homogeneous age subsets among sites, age structure of gray triggerfish may be considered similar across all sites in this study. Ages of fish sampled were generally restricted to the younger third (1-5 years) of the known age range (i.e., 0-15 years) and comprised only half of the ages (0-10 years) typically observed at the reefs sampled throughout the Gulf of Mexico (SEDAR, 2015). This feature is not observed at other artificial reef sites in the Gulf of Mexico (Ingram, 2001; Lang & Fitzhugh 2015).

## Growth

### von Bertalanffy Length-at-Age

Gray triggerfish on artificial reefs in the northwest Gulf of Mexico grow rapidly from < 1 to ~ 1.5-2.0 years of age before growth slows and maximum mean size is achieved for the region (Figure 5). However, the value of the variables  $L_{\infty}$  and  $k$  from the von Bertalanffy growth curves generated in this study are inconsistent with previous studies of this species (Johnson & Saloman, 1984; Escorriola, 1991; Ingram, 2001; Moore, 2001; Bernardes, 2002; Burton et al., 2014; Kacem et al., 2015b). The values observed in this study were influenced by a lack of individuals of age > 5 and fish which did not grow to larger sizes at higher ages. A highly variable FL at age was observed in the sampled population, with average FL being less than the previous age assignment in 14 of 24 (58%) occasions.

This study did not force  $t_0$  through zero, as it produced FL-at-age estimates which were inconsistent with the observed dataset. It is of note that von Bertalanffy length-at-age equations



used for comparisons among studies engaged in this practice (Johnson & Saloman, 1984; Escorriola, 1991; Bernardes, 2002; Kacem et al., 2015b).

Observed population FL-at-age equations could not be used here to represent growth throughout the lifespan of gray triggerfish because of the absence of larger and older fish. When the FL-at-age of fish < 1 to 5 years of age was estimated at 1.0-year intervals and compared to the FL-at-age findings reported in previous studies (Johnson & Saloman, 1984; Escorriola, 1991; Moore, 2001; Ingram, 2001; Bernardes, 2002; Burton et al., 2014; Kacem et al., 2015b) (Table 6) no significant difference could be observed among studies. Post-hoc testing revealed populations which were the least similar when collected from the geographical region most distant from this study (Kacem et al., 2015b). When Gulf of Mexico-exclusive studies are compared (Figure 7), no significant differences are evident among studies and post-hoc testing revealed the populations which were the least similar in length-at-age were sampled from the greatest geographical distance from this study (Escorriola, 1991).

The absence of individuals older than age 5 and larger than ~ 420 mm was of interest as individuals both older and larger were observed in previous studies conducted on artificial reefs in the Gulf of Mexico (Johnson & Saloman, 1984; Ingram, 2001; Burton et al., 2014). It is unknown at this time if the structure and distribution of the habitats surveyed in previous studies contributed to observed differences in populations of gray triggerfish.

Differences in collection methodologies are likely to have contributed to this outcome; of particular note was the absence of commercial fishery catch; however, the absence of statistical differences in length at ages < 1-5 suggest that methodology alone does not account for the purported size and age bias reported here.

## **Reproductive Status**

### **Reproductive Characterization**

Gray triggerfish at all life stages are observed on artificial reefs throughout the Gulf of Mexico (MacKichan & Szedlmayer, 2007; Simmons & Szedlmayer, 2011; Allman et al., 2017) and the identification of spawning-capable and spawning-active individuals (male and female) at sites sampled in this study through histological characterization confirms that artificial reefs in the northwest Gulf of Mexico are suitable habitat for gray triggerfish throughout their ontogeny.

Female fish were found at peak GSI just prior to the onset of spawning, as supported by histological observations of this study. A July GSI peak of females mirrors other studies of gray triggerfish from the Gulf of Mexico (Kacem et al. 2015b; Lang & Fitzhugh, 2015;). Average female GSI observed throughout the peak spawning season in this study was  $> 3$ , similar values were also reported by Lang & Fitzhugh (op. cit.). A peak GSI was also observed in July for male fish during; however, the GSI change for males is small (0.1-1.05) as compared to females (0.3-11). Most females were identified as either spawn capable or actively spawning during the month of July here and by others (Lang & Fitzhugh, op. cit.). Reproductive characters have been previously quantified for gray triggerfish on artificial reefs in the Gulf of Mexico with findings similar to those reported here (Kjesbu, 2009; Lang & Fitzhugh, op. cit.).

### **Maturity**

Fork length at 50% maturity of females in this study was different than reported by other studies. For example, Kacem et al. (2015a) observed that female size at maturity had  $\sim 200$  mm FL and males  $\sim 210$  mm FL. Ismen et al. (2004) calculated 50% maturity size as  $\sim 130$  mm FL

for both sexes. Fitzhugh et al. (2015) reported 50% female maturity at ~ 159 mm FL which was markedly different from Ismen et al. (2004), Kacem et al. (2015a) and this study.

Differences in FL at maturity may be attributed to a shortage of < 200 mm FL individuals in this study's samples and perhaps to geospatial differences in the areas studied. No females below 230 mm FL were collected in this study while in all other studies the sampled size range included individuals having < 200 mm FL, this is due in part to differences in sampling method which included a lack of access to commercial (trawl) efforts which readily collects larval and post larval fishes. Notably, all female gray triggerfish collected in this study were larger than the  $FL_{50}$  calculated; however, this does not imply that all females collected exhibited maturity, only that greater than 50% of collected individuals had reproductive histology which reflected characteristics of maturity.

Also, of note is the geographic area of study for Ismen et al. (2004) and Kacem et al. (2015b) which were performed in the Mediterranean Sea, while Fitzhugh et al. (2015) and this study were performed in the northern Gulf of Mexico. Life history estimations from multiple studies suggest regional discrepancies in growth rates are observed among gray triggerfish populations (Allman et al., 2017). These differences in growth can affect maturation and are a likely source of variation among studies.

### **Batch Fecundity**

Females selected to calculate batch fecundity as a function of FL were similar in FL to those examined by Ingram (2001) and Lang & Fitzhugh (2015); however, batch fecundity as a function of FL estimates, were found to differ statistically among studies ( $F_{(3,99)} = 5.360$ ,  $p = 0.01$ ). Post-hoc testing identified this study's estimations, which shared an equation format (i.e.,

linear or power functions), not to be significantly different from equations of the same structure of Ingram (2001) or Lang & Fitzhugh (2015). Deviations among batch fecundity as a function of length were observed between this study, Ingram (2001) and Lang & Fitzhugh (2015) due to differences in equation format. Batch fecundity as a function of weight was also significantly different among studies ( $F_{(2,99)} = 4.378$ ,  $p = 0.013$ ). Post-hoc testing revealed no statistical similarities irrespective of differences in equation structure when compared to the equation provided by Ingram (op. cit.).

Longer and heavier fishes produce a greater number of mature oocytes than smaller and lighter members of the same population (Hixon et al., 2014). While Ingram (2001) found age a fair predictor of batch fecundity in gray triggerfish, it was a poor indicator of batch fecundity for individuals collected on the artificial reefs off the coast of south Texas. This is due in part to FL and weight ranges which overlap several age groupings. Additionally, individuals above 435 mm FL, 1.3 kg weight, and age 5.5 years were not included in this study.

### **Population Structure and Implications**

Artificial structures and natural reefs throughout the Gulf of Mexico support populations of gray triggerfish with larger and older individuals. However, absence of fish > 435 mm FL and age > 5.5 years suggests that artificial reefs off the south Texas coast either do not support older, larger gray triggerfish or unidentified effects were responsible for the exclusion of larger and older fish at the sampled sites (Johnson & Saloman, 1984; Moore, 2001; Ingram, 2001; MacKichan & Szedlmayer, 2007; Herbig & Szedlmayer, 2016). This species distribution ranges from Nova Scotia to Argentina. Temperate and subpolar populations are exposed to a broader range of conditions which may contribute to selecting for subpopulation, for example, based on

size and age as reported here. Environmental variables are therefore unlikely to be the sole cause for the comparatively small age and size of gray triggerfish observed.

The frequency and intensity of fishing events occurring over artificial reefs in the northwest Gulf of Mexico sampled in this study is unknown; however, it is well established that the sampled sites are targeted year-round by commercial, recreational, and even illegal international and illegal local efforts (pers. obs.).

Texas Parks and Wildlife Department is responsible for setting legal fishing regulations in state waters, with minimum catch size set at 406 mm (16 in) FL with a daily bag limit of 20 as of January 1<sup>st</sup>, 2018 (TPWD, 2017). With the rare exception of relic natural hard bottom habitats, artificial reefs provide a relatively rare substrate for gray triggerfish in the northwest Gulf of Mexico (Mueller, 2012) easily accessible to commercial and recreational interests. Gray triggerfish are rarely the specific target of fishing efforts in the northwest Gulf of Mexico; however, they and other reef fishes are not strictly regulated in Texas state waters compared to other states around the Gulf of Mexico and federal waters (NOAA 2015; Alabama Department of Conservation and Natural Resources, 2017; Florida Fish and Wildlife Conservation Commission, 2017; Gulf of Mexico Fishery Management Council, 2017; Louisiana Department of Wildlife and Fisheries, 2017; Mississippi Department of Marine Resource, 2017; TPWD, 2017). While this study's population produced a growth curve which predicted gray triggerfish will rarely reach legal catch size, growth curves of previous studies identified individuals reaching legal catch (~ 405 mm FL) sizes among the ages of 4 to 6 years (Johnson & Saloman, 1984; Escorriola, 1991; Ingram, 2001; Moore, 2001; Bernardes, 2002; Burton et al., 2014; Kacem et al., 2015b), the same age range that is rare or absent from this study.

The US Federal fishery for gray triggerfish was in moratorium as of December 2017 and, while this measure was intended to bolster the stock, the majority of artificial reefs in south Texas reside in state waters (TPWD, 2016) and were open to fishing. While the impact of Texas fishery management of gray triggerfish is difficult to assess at this time, gray triggerfish recreational fisheries have been closed or highly regulated off all other US states bordering the Gulf of Mexico with the exception of Mississippi (Alabama Department of Conservation and Natural Resources, 2017; Florida Fish and Wildlife Conservation Commission, 2017; Gulf of Mexico Fishery Management Council, 2017; Louisiana Department of Wildlife and Fisheries, 2017; Mississippi Department of Marine Resources, 2017; TPWD, 2017).

While anthropogenic factors cannot be the exclusive cause for population dynamics (predation, habitat availability, resource availability, and recruitment are poorly studied in this species) on the artificial reefs in the northwest Gulf of Mexico, the trends reported here have a worrying implication for fishery management.

### **Fishery Management Implications**

Previous studies of the early life history and genetic distribution of larval gray triggerfish reported that larvae are capable of traveling great distances (> 4,000 km) before recruitment (Antoni et al., 2011; Antoni & Saillant, 2017) and a local retention to reef substrates (Simmons & Szeldmayer, 2011). The magnitude of local larval retention is unknown; genetic relationships examined in the Gulf of Mexico (Antoni & Saillant, op. cit.) demonstrated that western Gulf of Mexico gray triggerfish populations provide a source of larvae and juveniles fish which travel along the Mexican current and Gulf Loop current to northern and northeastern regions (Antoni & Saillant, op. cit.). This dispersal pattern suggests that impacts to gray triggerfish populations

from the northwest and south Texas can affect the overall population dynamics in the Gulf of Mexico.

Implications for reproductive capacity and selective genetic drift are of utmost concern in age-truncated populations. Gray triggerfish reach sexual maturity by age 1 or 2 years (Lang & Fitzhugh, 2015), and that larger fish produce more oocytes has been established (Hixon et al., 2014), but whether older gray triggerfish produce a greater number of oocytes irrespective of size remains unknown. While gray triggerfish in this region have been found to utilize artificial reefs during spawning events, if and how fishing affects spawning in this region remains to be determined. Additionally, gray triggerfish from this study's populations display a large overlap in size across age bins of one year. In 30% of cases individuals are 100 mm different in length at the same age. The observed variation in size at age suggested that individuals that reach large (> 400 mm FL) sizes at younger ages (< 4 years) may be targeted by fishing efforts and any individual which grows quickly would not be provided the opportunity to produce offspring. Without the input of large quick growing gray triggerfish there is potential for a decrease in mean size across the region.

If results from this study are representative of the population structure in the study region, given that older (larger) fish produce more mature oocytes than younger (smaller) fish implications exist for the reproductive capacity of the population. Absence of older, and by proxy larger fish conditions stock replenishment on a smaller and less fecund proportion of the gray triggerfish population.

Gulf-wide populations of gray triggerfish have been in decline, regardless of stock rebuilding plans enacted in the early 2000s, and as a result new restriction on the fishery have been set in a number of Gulf of Mexico state waters and federal waters (Gulf of Mexico Fishery

Management Council, 2018). Artificial reefs have been identified as fishery stock enhancement tools (Bombace et al., 1994; Bortone et al., 1994; Streich, 2017); however, without adequate management the beneficial increase in biomass afforded by these structures is negligible because of potential impacts by local recreational and commercial fishing efforts.



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## APPENDIX A

## APPENDIX A

### TABLES

Table 1. Histological characterization of A) ovaries and B) testes of gray triggerfish. Modified from Kacem et al. (2015a). A) Cell types females: Cortical alveolar = CA; Primary vitellogenic oocytes = Vtg1; Secondary vitellogenic oocytes = Vtg2; Tertiary vitellogenic oocytes = Vtg3; Postovulatory follicles = POFs; B) males: Germinal epithelium = GE; Primary spermatocyte = Sc1; Secondary spermatocyte = Sc2; Primary spermatogonia = Sg1; Secondary spermatogonia = Sg2; Spermatid = St; Spermatozoa = Sz.

A) Maturity (Females)	Microscopic characteristic
I. Immature	Well-defined cellular organization with the presence of several unyolked oocytes in perinuclear regions. Only oogonia and primary growth.
II. Developing	Oogonia, CA, Vtg1 and Vtg2. No evidence of Vtg3.
III. Spawning capable	All oocyte stages present and abundance of Vtg3.
IV. Actively spawning	Hydrated oocytes (translucent) in large quantities and with few POFs.
V. Regressing	Few POFs, atresia (any stage), some residual CA and Vtg1-Vtg2 oocytes.
VI. Resting	Reorganization of unyolked oocytes to the beginning of a new reproductive cycle, and atresia.
B) Maturity (Males)	Microscopic characteristics
I. Immature	Small testes, often clear and threadlike. Only Sg1 present; no lumen in lobules.
II. Developing	Small testes but easily identified. Spermatocysts evident along lobules. Sg2, Sc1, Sc2, St, and Sz can be present in spermatocysts. GE continuous throughout.
III. Spawning capable	Large and firm testes. Sz in lumen of lobules and/or sperm ducts. All stages of spermatogenesis can be present. GE can be continuous or discontinuous.
IV. Regressing	Small and flaccid testes, no milt release with pressure. Residual Sz present in lumen of lobules and in sperm ducts. Widely scattered spermatocysts near periphery containing Sc2, St, Sz. Spermatogonial proliferation and regeneration of GE common in periphery of testes.
V. Resting	Small testes, often threadlike. No spermatocysts. Lumen of lobule often absent. Proliferation of spermatogonia throughout testes. GE continuous throughout.



Table 2: Fork length (mm) of gray triggerfish collected from three artificial reefs in the northwest Gulf of Mexico. All lengths reported in mm; n = 112; PI = PS-1169L (Port Isabel Reef), PM = PS-1047 (Port Mansfield Reef), LB = PS-1070 (Liberty Ship Reef), Max = maximum, Min = minimum, STD = standard deviation.

Site	Sex	Mean	Max	Min	STD
PI	Female	284	375	235	29
	Male	320	374	262	37
	Total	296	375	235	36
PM	Female	307	345	271	22
	Male	324	380	244	30
	Total	319	380	244	29
LB	Female	328	432	232	44
	Male	355	405	297	30
	Total	338	432	232	41
All Sites	Female	302	432	232	39
	Male	329	405	244	34
	Total	315	432	232	39

Table 3: Weight (g) of gray triggerfish collected from three artificial reefs in the northwest Gulf of Mexico. Abbreviations and sample size as in Table 2.

Site	Sex	Mean	Max	Min	STD
PI	Female	572.1	1,122.1	322.1	167.8
	Male	829.9	1,265.2	443.3	270.9
	Total	658.1	1,265.2	322.1	238.8
PM	Female	684.5	1,033.8	465.0	155.8
	Male	797.4	1,258.5	349.0	194.1
	Total	763.8	1,258.5	349.0	188.8
LB	Female	878.7	1,564.8	302.9	292.6
	Male	1078.1	1,501.8	586.4	261.4
	Total	951.8	1,564.8	302.9	293.7
All Sites	Female	689.8	1,564.8	302.9	250.2
	Male	866.2	1,501.8	349.0	254.0
	Total	771.9	1,564.8	302.9	265.9

Table 4. Age (years) of gray triggerfish collected from three artificial reefs in the northwest Gulf of Mexico. Abbreviations and sample size as in Table 2.

Site	Sex	Mean	Max	Min	STD
PI	Female	2.6	5.2	0.7	1.1
	Male	2.4	4.2	1.0	0.8
	Total	2.5	5.2	0.7	1.0
PM	Female	2.3	4.3	1.1	1.0
	Male	2.2	5.1	0.7	1.0
	Total	2.3	5.1	0.7	1.0
LB	Female	2.8	4.2	1.1	1.1
	Male	3.0	4.2	2.1	0.8
	Total	2.9	4.2	1.1	1.0
All Sites	Female	2.6	5.2	0.7	1.1
	Male	2.4	5.1	0.7	0.9
	Total	2.5	5.2	0.7	1

Table 5. A) Power fit and B) linear fit of weight (g) as a function of fork length (mm) for gray triggerfish collected from three artificial reefs in the northwest Gulf of Mexico.

A) Power			
Sex	Equation	r <sup>2</sup>	p
Males	$Wg = 6.40 \times 10^{-5} \times FL^{2.8}$	0.94	0.001
Females	$Wg = 1.54 \times 10^{-4} \times FL^{2.7}$	0.93	0.001
Combined	$Wg = 1.07 \times 10^{-4} \times FL^{2.7}$	0.94	0.001
B) Linear			
Males	$Wg = 7.1 \times FL - 1459.8$	0.94	0.001
Females	$Wg = 6.2 \times FL - 1189.7$	0.93	0.001
Combined	$Wg = 6.6 \times FL - 1290.1$	0.93	0.001

Table 6. Three parameter length-at-age von Bertalanffy growth curves generated for gray triggerfish calculated from this and previous studies. Where  $L_{\infty}$  = mean asymptotic length where growth = 0,  $k$  = growth rate,  $t_0$  = age where length = 0, region = geographic region of specimen collection, combined = both male and female individuals included.

Study	Region	Sex	$L_{\infty}$	$k$	$t_0$
This Study	Gulf of Mexico	Combined	326	0.9	-1.706
		Males	341	0.9	-1.606
		Females	326	0.9	-1.706
Kacem et al. (2015b)	Mediterranean	Males	521	0.23	0.12
		Females	417	0.24	0.07
Burton et al. (2014)	Gulf of Mexico	Combined	457	0.33	-1.58
Bernardes (2002)	Brazil	Males	519	0.26	-0.04
		Females	505	0.27	0.03
Ingram (2001)	Gulf of Mexico	Combined	583	0.18	-1.6
Moore (2001)	Gulf of Mexico	Males	521	0.17	2.03
		Females	443	0.19	2.26
Escorriola (1991)	Southeastern United States	Combined	571	0.19	-0.15
Jonhson & Saloman (1984)	Gulf of Mexico	Combined	466	0.38	-0.19

Table 7. A) Observed batch fecundity of gray triggerfish, n = 12. B) Calculated batch fecundity as a function of fork length (mm) or total weight (g) for gray triggerfish collected from three artificial reefs in the northwest Gulf of Mexico. Max = maximum, Min = minimum, STD = standard deviation, n = 56.

A) Observed	Max	Min	Mean	STD
	$1.11 \times 10^6$	$3.39 \times 10^5$	$7.36 \times 10^5$	$2.69 \times 10^5$
B) Calculated				
Fork Length	$1.31 \times 10^6$	$4.06 \times 10^5$	$6.77 \times 10^5$	$2.27 \times 10^4$
Weight	$1.31 \times 10^6$	$4.06 \times 10^5$	$6.77 \times 10^5$	$2.27 \times 10^4$

Table 8. Batch fecundity as a function of morphometrics of gray triggerfish from three studies conducted in the Gulf of Mexico and abroad. Batch fecundity = BF, fork length = FL (mm), weight = Wg (g).

Fork Length (FL)	Equation	r <sup>2</sup> value
This study	BF = 15 x FL <sup>1.88</sup>	0.27
	BF = 3,691 x FL - 378,243	0.17
	Log BF = 2.79 x Log (FL <sup>0.81</sup> )	0.28
Lang & Fitzhugh (2015)	BF = 8,704 x FL - 1,776,483	0.56
Ingram (2001)	BF = 13,573 e <sup>(0.01 (FL))</sup>	0.72
Total Weight (Wg)		
This study	BF = 5,074 x Wg <sup>0.76</sup>	0.30
	BF = 685 x Wg + 274,252	0.18
	Log BF = 3.994 x Log (Wg <sup>0.37</sup> )	0.40
Ingram (2001)	BF = 836 x Wg - 202,904	0.83

## FIGURES

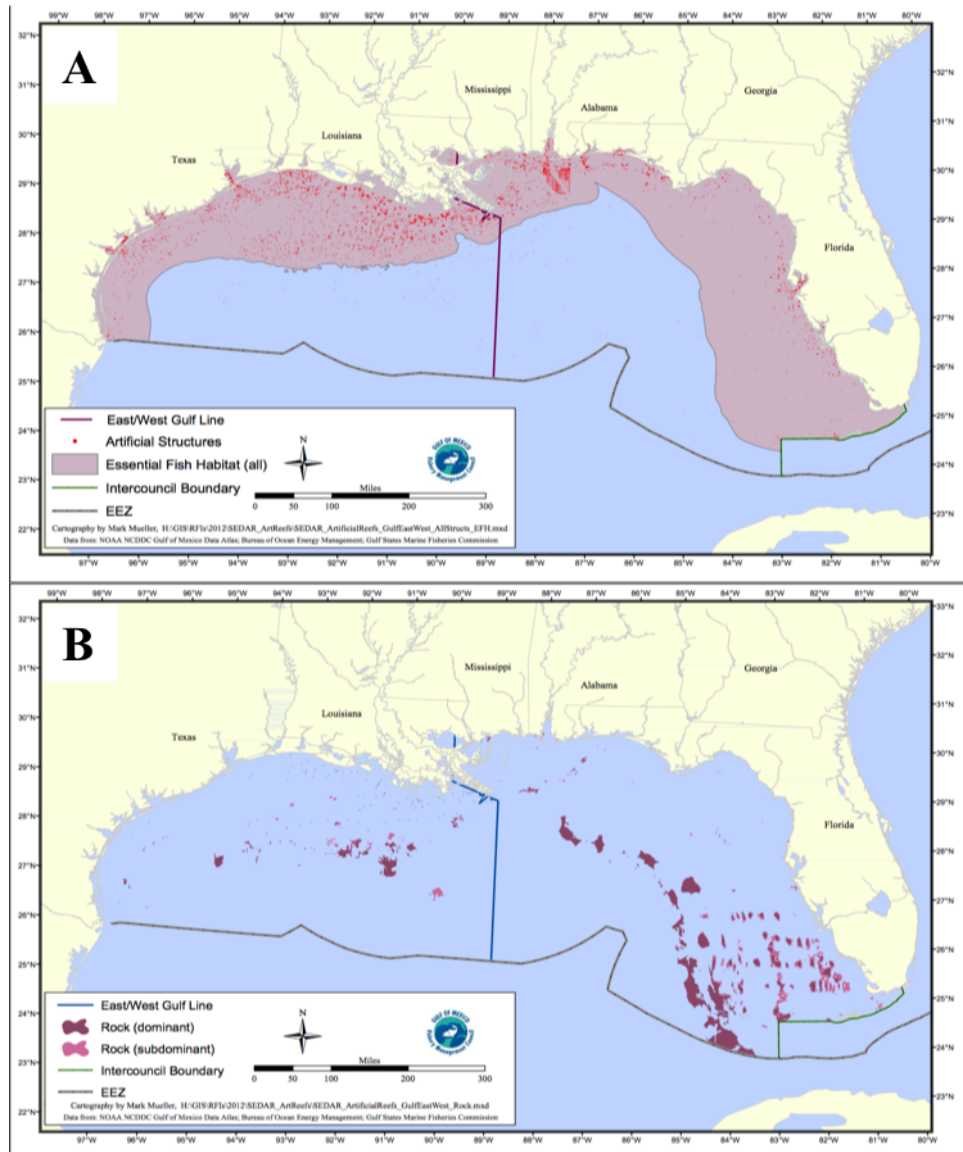


Figure 1. A) Artificial structures distribution in the Gulf of Mexico. B) Hard bottom habitat availability in the Gulf of Mexico. Images adapted from Mueller (2012).



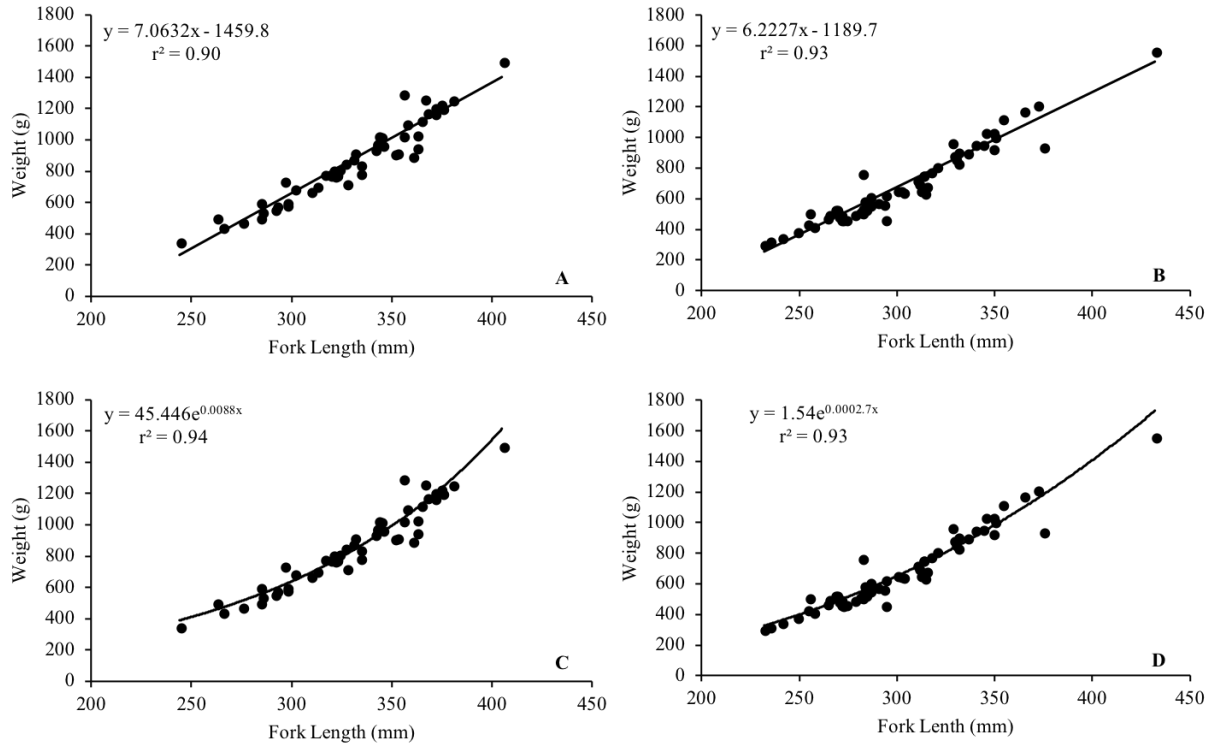


Figure 2. Weight (g) as a function of fork length (mm) for gray triggerfish collected from three artificial reefs in the northwest Gulf of Mexico. A) Linear fit males, B) linear fit females, C) power fit males, D) power fit females. Sample size = n, n males = 56, n females = 56.

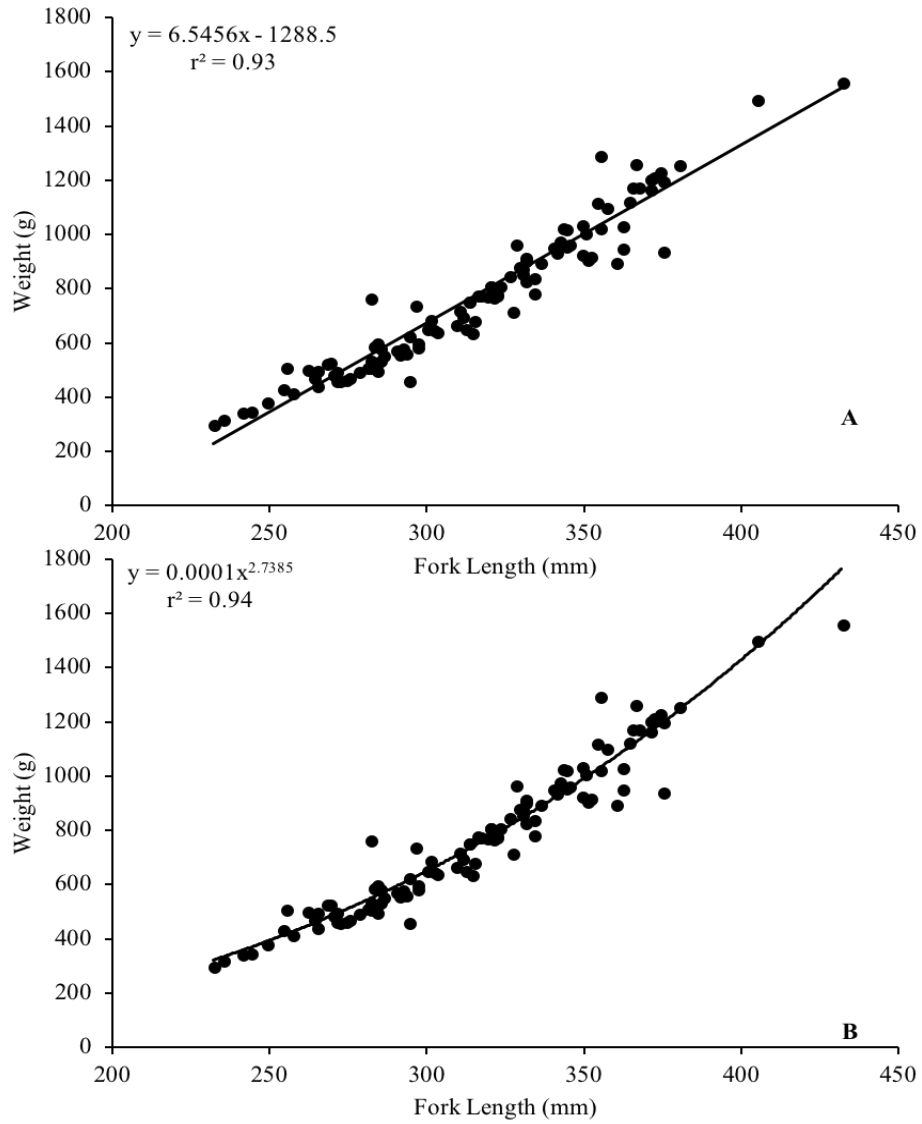


Figure 3. Weight (g) as a function of fork length (mm) for gray triggerfish collected from three artificial reefs in the northwest Gulf of Mexico. A) Linear fit. B) Power fit. n =112.

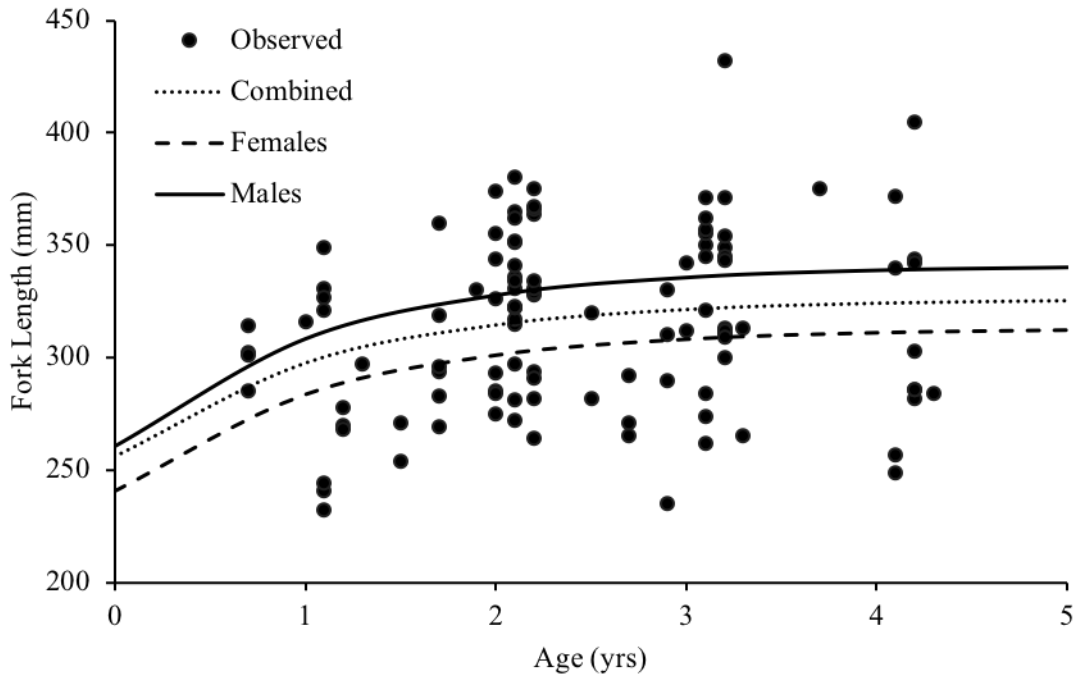


Figure 4. Observed fork length-at-age and the associated three parameter von Bertalanffy length-at-age growth curves for gray triggerfish collected from three artificial reefs in the northwest Gulf of Mexico. For equations from growth curves, see Table 6 n = 112.

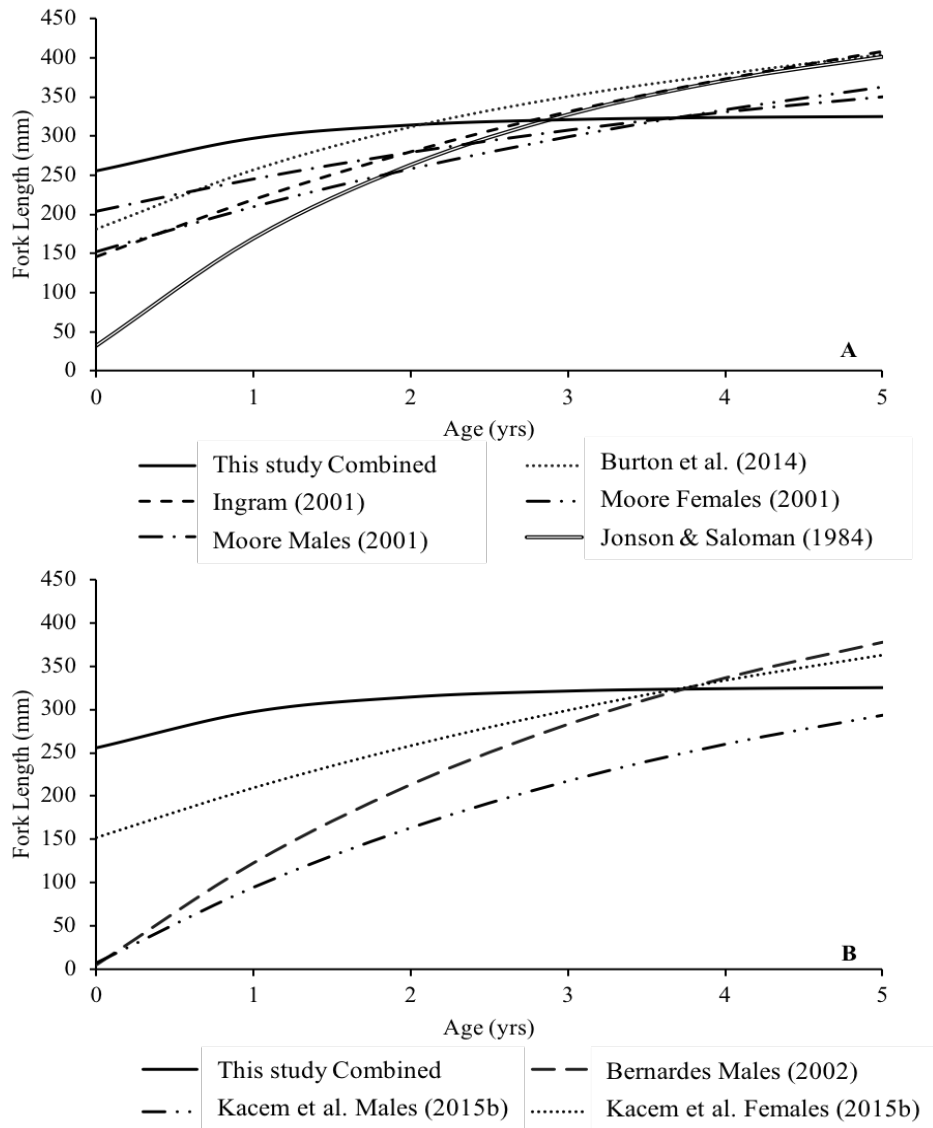


Figure 5: Length-at-age von Bertalanffy growth curves for gray triggerfish for ages 0-5 years. A) Studies from the Gulf of Mexico and this study. B) Studies from international populations and this study for equation details see Table 6.

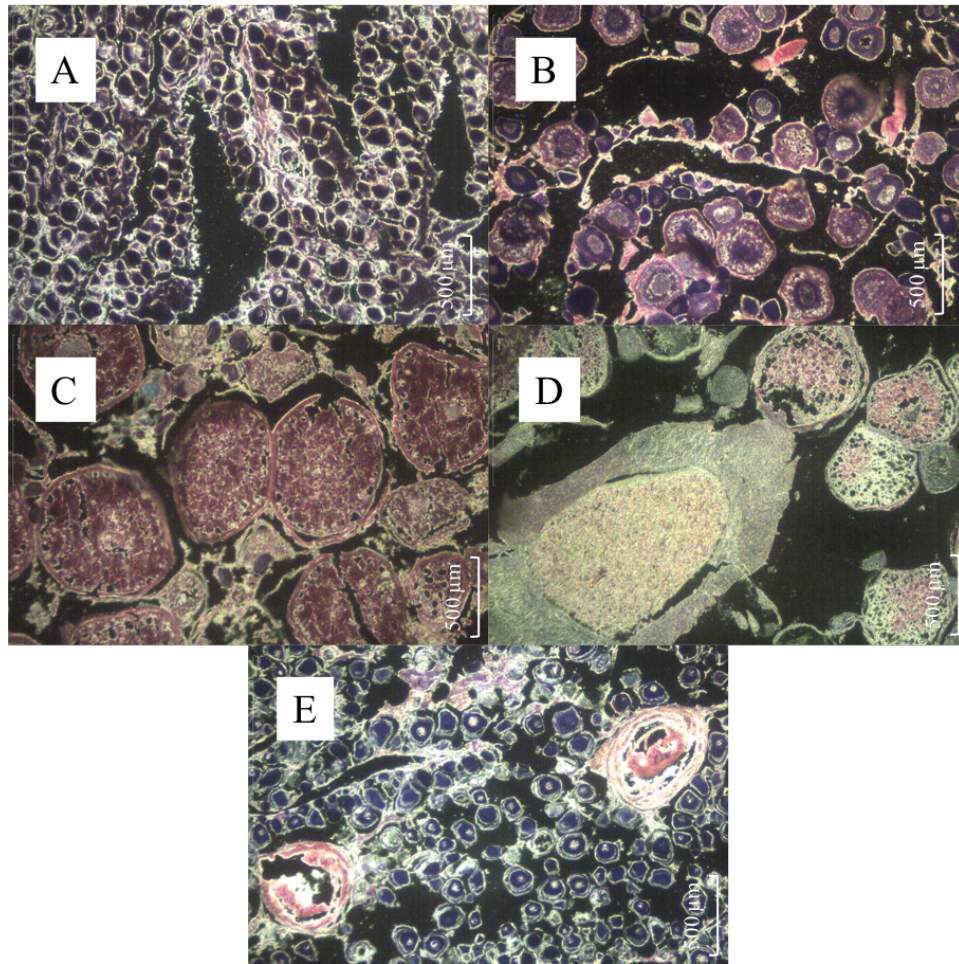


Figure 6. Ovarian stages of gray triggerfish collected from three artificial reefs in the northwest Gulf of Mexico. A) Immature or resting, B) developing, C) spawning capable, D) active spawning, and E) regressing. For descriptions of tissues see Table 1.

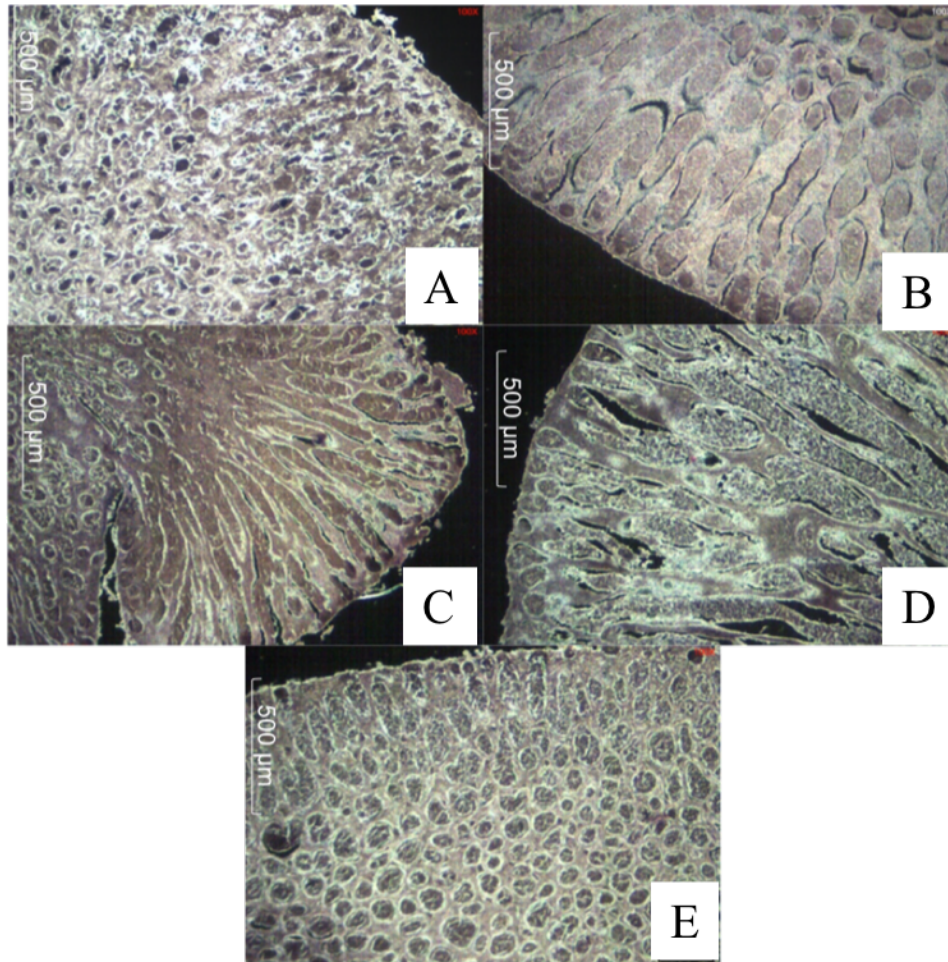


Figure 7. Testis stages of gray triggerfish collected from three artificial reefs in the northwest Gulf of Mexico. A) Immature or resting, B) developing, C) spawning capable, D) active spawning, and E) regressing. For descriptions of tissues see Table 1.

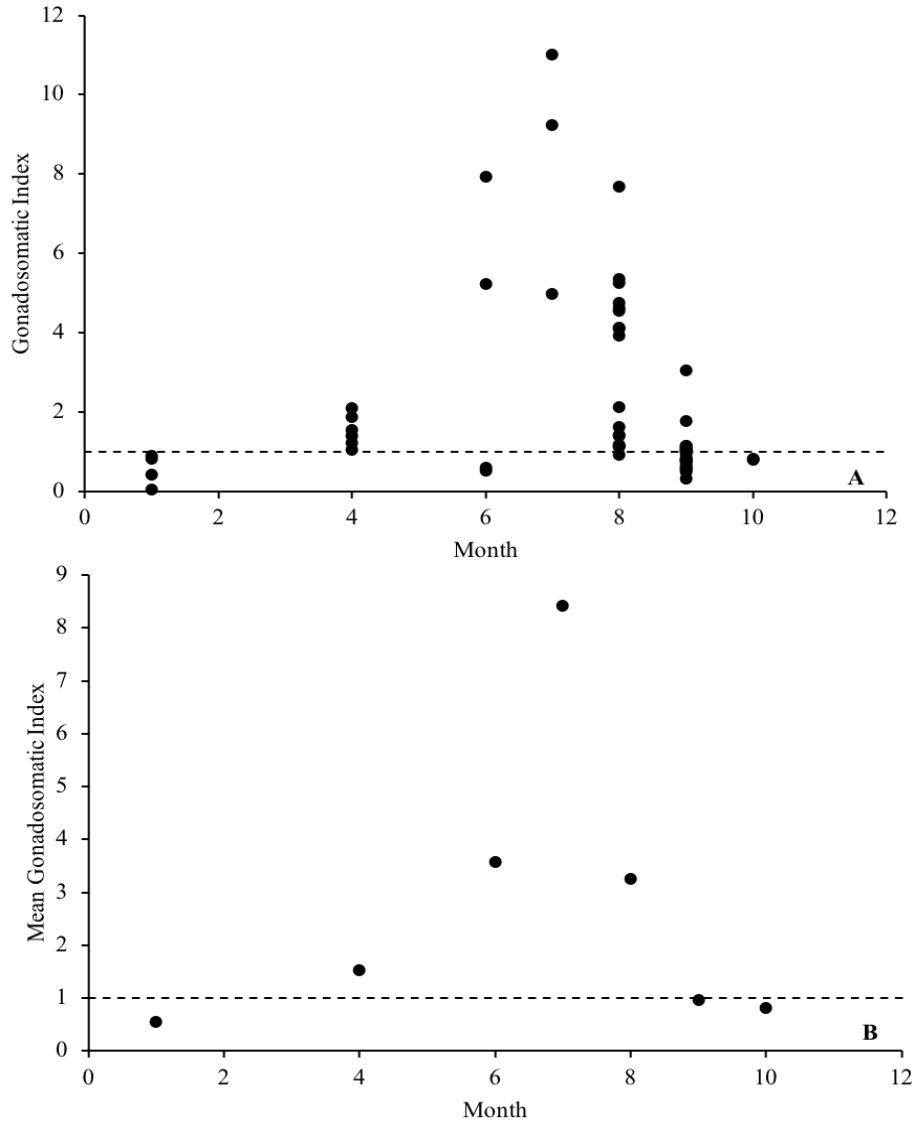


Figure 8. Gonadosomatic index (GSI) of female gray triggerfish on artificial reefs in the northwest Gulf of Mexico by A) month captured throughout the sampled year and B) mean GSI by month. Dashed line represents GSI = 1, individuals expressing an index above 1 are considered spawn capable; n = 56.

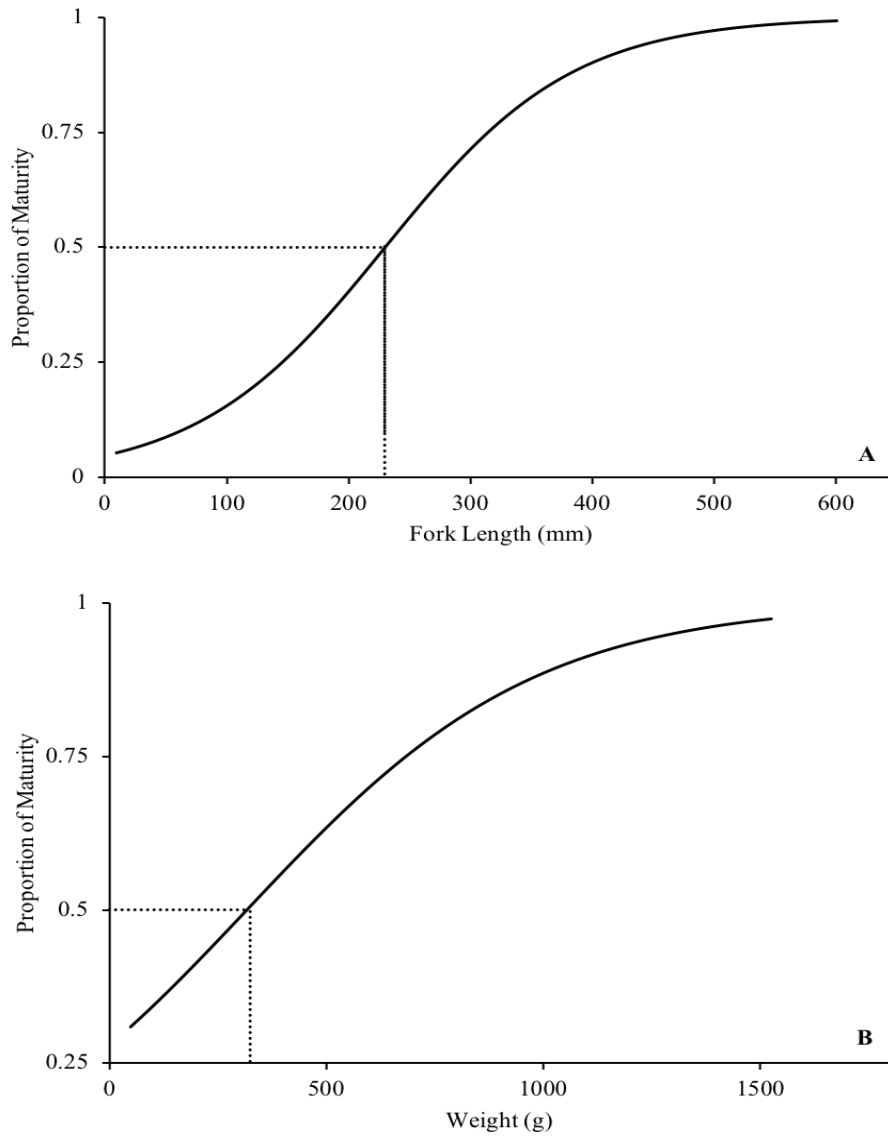


Figure 9. Proportional maturity for female gray triggerfish collected from three artificial reefs in the northwest Gulf of Mexico, A) at fork length (mm) where proportion mature =  $1/1 + e^{(-2.999 + 0.013 * FL)}$ ; B) at weight (g) where proportional maturity =  $1/1 + e^{(-0.955 + 0.003 * Wg)}$ , n = 56.



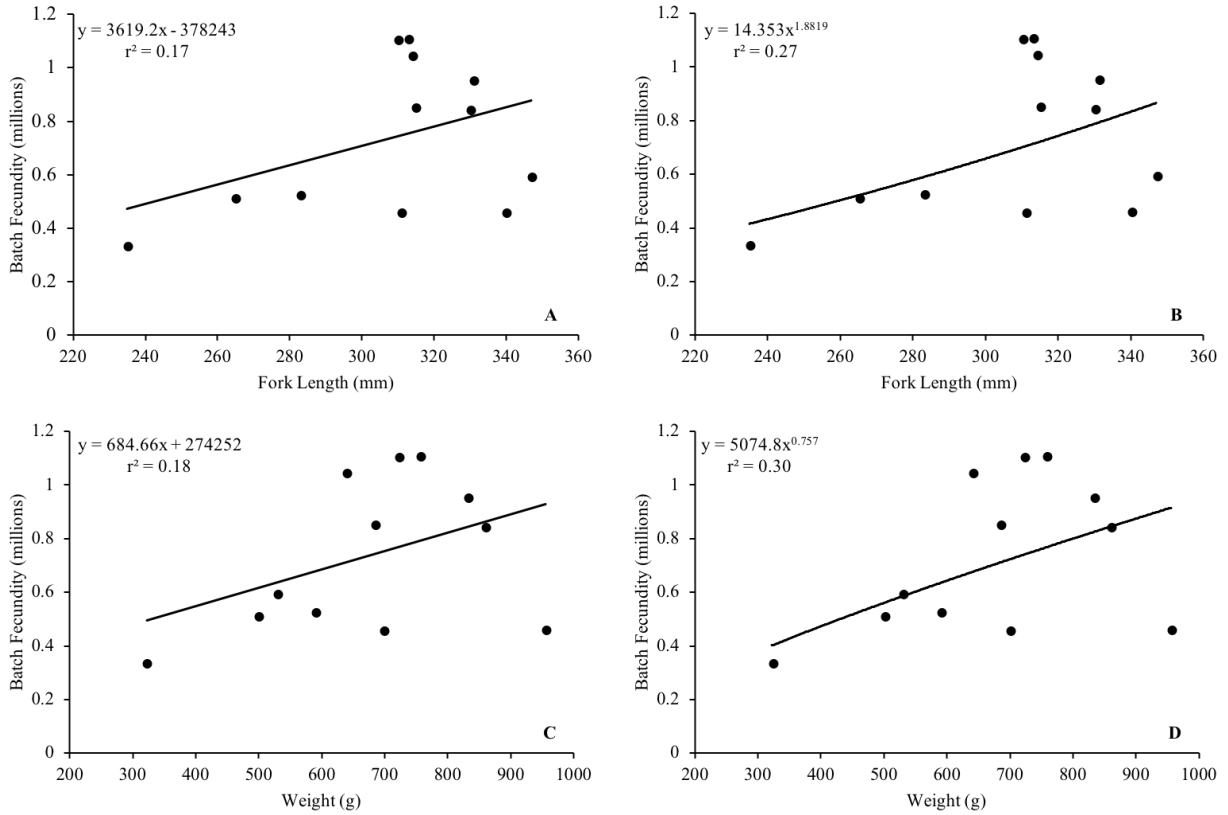


Figure 10. Log-transformed batch fecundity as a function of log transformed A) Fork length (mm) and B) weight (g) for gray triggerfish collected from three artificial reefs in the northwest Gulf of Mexico,  $n = 12$ .

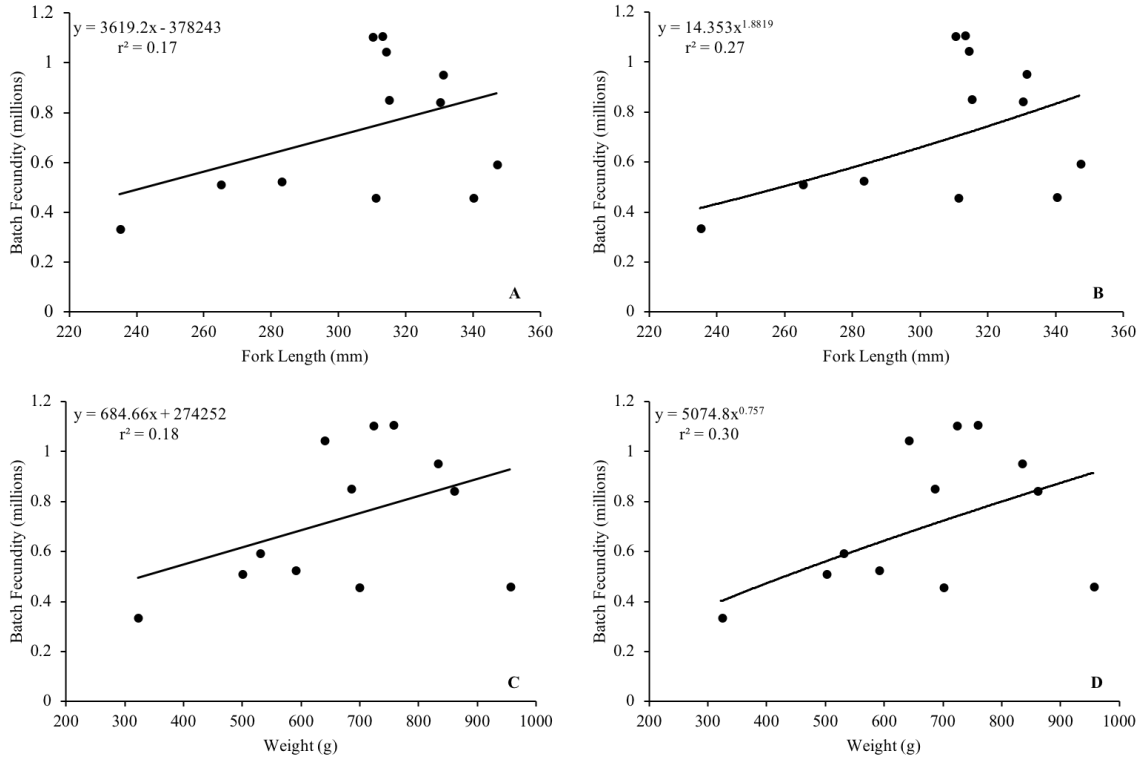


Figure 11. Mean batch fecundity ( $1 \times 10^6$  oocytes) as a function of A) fork length (mm) linear fit and B) fork length (mm) power fit. C) Weight (g) linear fit and D) weight (g) power fit for gray triggerfish collected from three artificial reefs in the northwest Gulf of Mexico,  $n = 12$ .

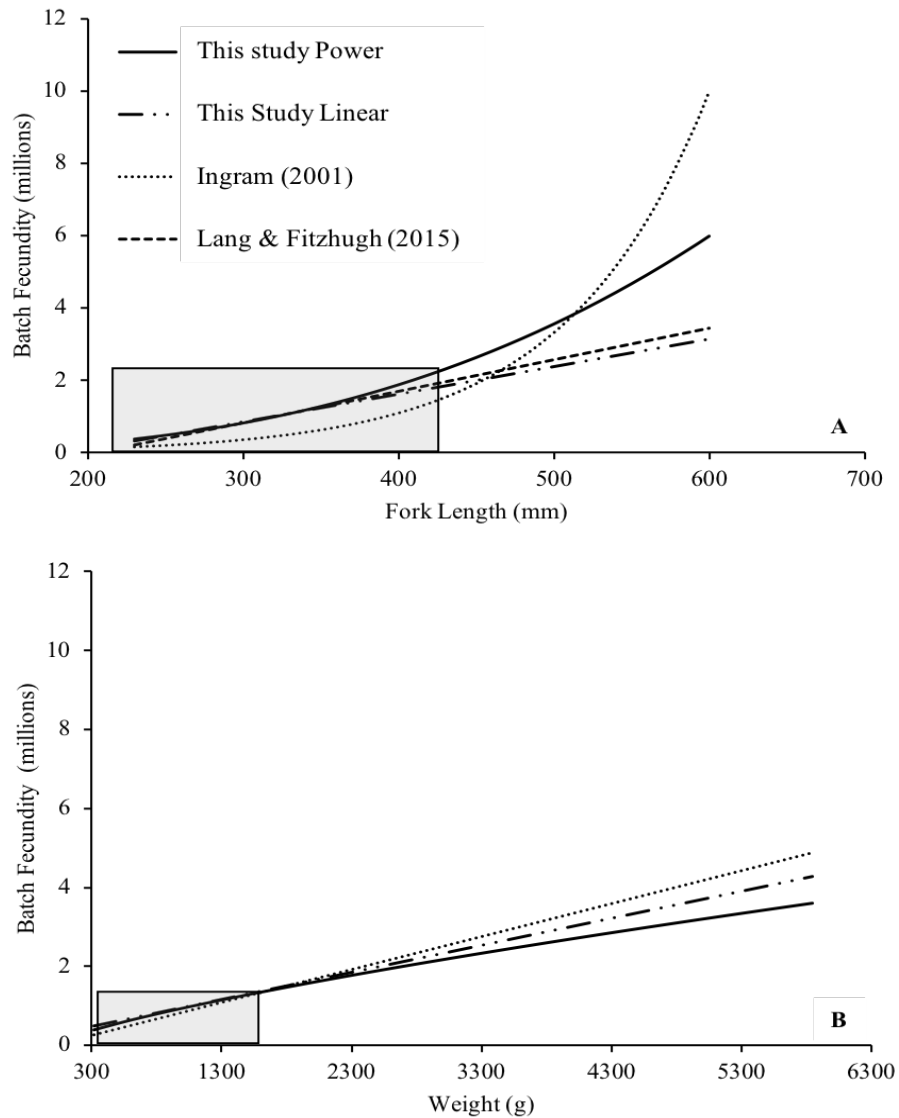


Figure 12. Batch fecundity ( $1 \times 10^6$  oocytes) from three studies of gray triggerfish in the Gulf of Mexico, A) as a function of fork length (mm), gray box represents observed size range from this study; B) as a function of weight (g), gray box represents observed weight range from this study. For batch fecundity equations see Table 8.

## BIOGRAPHICAL SKETCH

Adam M Lee was raised in the historic seaport town of Newburyport Massachusetts by his loving parents Michael Lee and Pamela Procter Lee alongside his twin sister Courtney. He spent his formative years absorbed in the areas rich maritime environments and developed a love of the ocean and coastal habitats. He left the area after high school to pursue a B.S. in Marine Science and Chemistry at Eckerd College in St Petersburg, Florida.

His undergraduate career fostered an interest in reproductive physiology and ecology which he applied during his time as an andrology and histology technician in a series of human infertility clinics. Human reproductive medicine provided invaluable experiences but was not his ultimate destination.

The University of Texas Rio Grande Valley's School of Earth, Environmental, and Marine Sciences provided a unique opportunity to investigate his two great interests in the heart of south Texas.

Under the supervision of Texas Parks and Wildlife Artificial Reef Program and Dr. Carlos Cintra-Buenrostro he investigated reef fish life history while improving his Spanish and finding lifelong companions. He completed his Master's of Science in May 2019.

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