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Does Reproductive Potential of Red Snapper in the Northern Gulf of Mexico Differ Among Natural and Artificial Habitats?

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DOES REPRODUCTIVE POTENTIAL OF RED SNAPPER IN THE
NORTHERN GULF OF MEXICO DIFFER AMONG NATURAL AND
ARTIFICIAL HABITATS?

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Oceanography and Coastal Sciences

by
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B.S., University of California at Santa Cruz, 2007
December 2014

This work is dedicated to my mother and grandmothers who keep watch from Elysium and to my father who taught me to fish (and had to rush me to the doctor when I had a fish hook stuck in my cheek).

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ABSTRACT

This study compares the reproductive potential of red snapper (*Lutjanus campechanus*) at artificial and natural habitats. Natural habitats are areas of vertical relief created from biogenic/geologic processes that are thought to be the historical centers of abundance for red snapper in the northern Gulf of Mexico. Red snapper were collected from 2011 to 2013 at three natural habitat sites and two artificial habitat oil and gas platform sites. The mean gonadosomatic indices (GSI), maturity at size and age, spawning indicators, batch fecundity, spawning frequency, and annual fecundity for red snapper at each habitat were analyzed to examine reproductive potential among sites and habitats. Results indicate red snapper on natural habitats exhibited higher mean GSI than those on artificial habitats during peak spawning season. Female red snapper on natural habitats showed 98% maturity, compared to 52% maturity of females on artificial habitats. These data support previous observation of demographic differences in red snapper reproduction between natural and artificial habitats. The differences observed may be due to red snapper at natural habitats being in better nutritional condition than red snapper on artificial habitats, thus allowing red snapper on natural habitats to invest more energy in reproduction and less energy towards rapid growth early in life.

CHAPTER 1: GENERAL INFORMATION

RED SNAPPER BIOLOGY

The red snapper (*Lutjanus campechanus*) inhabits coastal waters from the Yucatan peninsula to southern Florida in the Gulf of Mexico and along the south Atlantic US coast from Florida to North Carolina. There are two centers of abundance for red snapper in the northern Gulf of Mexico (NGOM), the waters off Alabama and Louisiana, split by the output of the Mississippi River (Patterson et al. 2001). Red snapper is a demersal, reef-associated species that is long-lived (over 50 years) and may grow larger than 1000 mm in total length (Patterson et al. 2001, Wilson and Nieland 2001, Wells 2007, Saari 2011). It exhibits rapid linear growth until reaching about 10 years of age, after which growth slows until a maximum length and weight are achieved (Patterson et al. 2001, Wilson and Nieland 2001).

Red snappers in the NGOM exhibit an ontogenetic shift in habitat use. As larvae, red snappers are most abundant in 50 to 100 m of water off the central and western coast of Louisiana (Gallaway et al. 2009). Juvenile red snappers settle on near-shore, low relief habitat, such as sand, shell rubble, or low-profile reefs (Workman and Foster 1994, Bailey 1995, Rooker et al. 2004). As red snapper grow older and larger, they move to areas with higher relief, such as oil and gas platforms or the shelf edge reefs (Patterson et al. 2001, Szedlmayer and Lee 2004, Wells 2007, Gallaway et al. 2009). After several years of residence at such habitats, they move off in small groups and spawn in deeper waters (Nieland and Wilson 2003).

Although red snapper can mature sexually as early as age 2, maximum fecundity is not reached until between ages 8 and 12, when somatic growth subsides (Render and

Wilson 1992, Collins et al. 1996, Woods et al. 2003, Gallaway et al. 2009). A female that reaches peak fecundity may produce between 60 and 70 million eggs per year (Collins et al. 1996, Woods 2003, SEDAR7 2005). Red snapper are gonochoristic broadcast spawners with the ability to spawn multiple times during a season (Grimes 1987, Winemiller and Rose 1992, Woods 2003, Woods et al. 2003). Spawning season in the NGOM is reported to last from May to September for approximately 150 days and peaks from June to August (Bradley and Bryan 1975, Render and Wilson 1992, Collins et al. 1996, Woods et al. 2003).

Previous studies in the NGOM off Louisiana have determined that female red snapper reach 50% maturity at 400-450 mm total length and 100% maturity above 700 mm total length (Woods 2003, Woods et al. 2003, Fitzhugh et al. 2004, Kulaw 2012). Red snapper also exhibit a diel spawning cycle in which the oocytes begin to hydrate during mid-morning and hydrated eggs are released by early evening (Jackson et al. 2005). Although there is a great deal known about regional differences in red snapper reproduction and life history, we know little about the potential impacts on reproductive variables of different habitats, especially natural habitats.

Red snapper reproduction is well described in the literature. The first description of red snapper reproduction was from a review of the family Lutjanidae (Grimes 1987). Grimes reviewed all the information available at the time, making broad statements about the Lutjanidae as a whole from information about specific species. Grimes stated that Lutjanidae are gonochoristic broadcast batch spawners that are normally highly fecund. Large females reach sexual maturity earlier than males of the same species and, in most

species, spawning seasons center around the summer; however, isolated tropical species may spawn year round (Grimes 1987).

Collins et al. (1996) conducted the earliest comprehensive study on red snapper reproduction with specimens collected from recreational and commercial landings in Panama City, Florida. Collins et al. (1996) were the first to report batch fecundity, spawning frequency, and annual fecundity for red snapper in the northeastern Gulf of Mexico. They established the first maximum fecundity estimates for red snapper and found that age 8 females tend to spawn more often than younger females. They concluded that more samples of older females were needed to obtain a better understanding of fecundity estimates (Collins et al. 1996).

Woods (2003) collected over 1,600 female red snapper and examined size and age at maturity, as well as, spawning periodicity and batch fecundity. She found significant differences in batch fecundity from females collected east and west of the Mississippi River. The spawning frequency analysis determined that 18% more female red snapper from Alabama are spawning on any given day during spawning season than red snapper females from Louisiana (Woods 2003). Woods (2003) also created the Index of Reproductive Importance (IRI), which takes into account annual fecundity at age data to determine reproductive importance at age by region. She found that female older than 8 years old are the most important spawning contributors in Louisiana (Woods 2003). In Alabama females older than 8 years were also the most important but 4 year olds were almost of equal importance (Woods 2003). Woods (2003) concluded that the red snapper population in the Gulf of Mexico is separated demographically and should not be managed as a unit stock.

Woods et al. (2003) investigated red snapper reproduction in the NGOM from east and west of the Mississippi River; all specimens were harvested from artificial habitats, i.e., low relief structure in Alabama and oil/gas platforms in Louisiana. They found that female red snapper in Alabama waters matured at younger ages and smaller sizes than female red snapper from Louisiana waters. No significant differences in the growth rate of red snapper between the two areas were observed. They concluded that size and age at maturity of red snapper may differ between distinct regions within the range of the species, due to possible environmental differences and fishing pressure (Woods et al. 2003).

Fitzhugh et al. (2004) sampled red snapper throughout the NGOM, although the majority of the specimens came from off the Florida panhandle. They found that red snapper reach 100% maturity at 650 mm fork length, both east and west of the Mississippi River. They also established that 75% maturity is reached at 300 mm FL east of the River and 350 mm FL west of the River. Additionally, a higher percent of age 2-7 female red snappers were observed east of the River. Fitzhugh et al. (2004) suggested that the fecundity at age relationships may be dome-shaped, although they do state this is an area for further examination.

Jackson et al. (2005) also examined female red snapper both from east and west of the Mississippi River and from different types of artificial habitat. This study specifically focused on the time of day red snapper oocytes undergo hydration and subsequent ovulation/spawning. Previous studies had speculated that red snapper hydrate in the early evening (Grimes 1987, Collins et al. 1996). Jackson et al. (2005) used hourly sampling to show that oocyte hydration starts in the morning, takes approximately five

hours to complete, and ovulation occurs no longer than five hours after hydration. Peak spawning was reported in the afternoon (1400 hours) (Jackson et al. 2005).

Brulé et al. (2010) examined red snapper reproduction at nearly submerged or emergent coral reef structures on the Campeche Banks in the southern Gulf of Mexico. Red snapper in spawning condition were found on both natural habitat sites sampled. They found size and age at maturity to be consistent with other studies of female red snappers in the NGOM, except that spawning season on the Campeche Banks (February to November) was more protracted than in the NGOM (Brulé et al. 2010).

Kulaw (2013) analyzed reproduction of red snapper from three habitats off Louisiana: Jakkula Reef (a natural shelf reef), standing oil and gas platforms, and toppled oil and gas platforms. All of the platforms were in the Eugene Island Artificial Reef Planning Areas. Kulaw (2013) found that gonadosomatic indices (GSI) were highest among female red snappers at Jakkula Reef. In addition, red snapper were heavier-at-age on Jakkula Reef than at the artificial habitat sites (Saari 2011). The previous studies identified demographic differences in reproductive potential for female red snappers between habitats. My project was designed to investigate these demographic differences further by analyzing the reproductive potential of female red snapper on shelf edge reefs that are further west of Jakkula and on toppled platforms in the East Cameron Artificial Reef Planning Area that is west of the Eugene Island sites.

This project sought to expand the knowledge of red snapper reproductive biology beyond artificial habitats by examining populations found at natural habitats found on the Louisiana/Texas continental shelf edge. These reefs create a network of naturally hard and vertically complex substrate. Long before oil and gas was discovered, and platforms

constructed in the NGOM, the reefs likely were the historical center of abundance for red snapper (Goodyear 1995).

REPRODUCTION AND FISHERY MANAGEMENT

Red snapper populations are vulnerable to overfishing because they are long lived and reach maximum reproductive output late in life (Gallaway et al. 2009). Due to high fishing pressure, many individuals are removed from the population before reaching full reproductive potential. Both egg size and duration of a spawning season can also fluctuate with fish body size (Trippel 1995, Jackson et al. 2007, Cowan et al. 2011). The quality of the eggs and larvae increases with parent body size, presumably because at larger sizes the parent is contributing more energy to reproduction and less energy to growth. Therefore, the removal of larger, older red snapper can affect the overall quality of eggs being produced by the population, which influences policy decisions because recovery of the NGOM red snapper stock is dependent upon a healthy spawning population.

Red snapper are economically important to Louisiana and other Gulf of Mexico states and support large recreational and commercial fisheries. The species' slow recovery since the collapse in the late 1980's has fueled contention about the pace and mechanisms of rebuilding the stock (Cowan 2011, Cowan et al. 2012). A healthy spawning population is essential to stock recovery, making information regarding reproduction crucial to assessing stock status (Trippel 1995, Collins et al. 1996, Woods 2003). Reproductive variables of red snapper are used in estimates of maximum sustainable yield – the amount of fish that can be removed by fishing while sustaining the

current stock size (Strelcheck and Hood 2007). Without considering the current population's reproductive capabilities, it is difficult to formulate a good prediction of the size of future populations and to gauge the numbers of fish available to the fisheries. Managers and stock assessment personnel generally have limited information about reproductive limits, even though it is an important part of the process.

Understanding red snapper reproduction is key to defining fisheries standards that allow the population to recover, as well as to promote economically successful and sustainable fisheries. If large, reproductively important females are being removed without allowing time for the population to replace them, the spawning capabilities of the remaining population could be adversely impacted. Gaining a better estimate of red snapper reproductive potential may improve their spawning success in future generations. This study seeks to enhance information about the potential reproductive output of populations of red snapper in the NGOM.

Red snapper management began in the late 1980s following a crash of the population in the NGOM. The original stock recovery was set for 2000 based upon a red snapper generation time of fifteen years; however the time to achieve rebuilding has been lengthened several times as estimates of longevity increased (up to 55 years) (Hood et al. 2007). As such, the rebuilding schedule has now been extended to the year 2032 (Hood et al. 2007, Strelcheck and Hood 2007). Since the inception of management for the red snapper fishery, it has been among the most controversial fisheries in the NGOM (Cowan 2011). This is partly due to the complicated life history of red snapper, but some controversy can be attributed to the large-scale addition of oil and gas platforms to the

NGOM over the last half-century and how red snapper utilize this de-facto artificial reef system.

Oil and gas platforms tend to attract structure-oriented fish species, like red snapper, and thus function as artificial reefs. Although there is no doubt that oil and gas platforms shelter higher numbers of fish than surrounding flat mud habitats, the functional value of these structures is debated (Bohnsack 1989, Cowan 2011, Harwell 2013, Simonsen 2013). Are red snapper reproductive characteristics similar at natural and artificial habitats? Recent data indicate that red snapper found on natural habitat may be physiologically and demographically different from the populations that inhabit the oil and gas platforms (Brulé et al. 2010, Saari 2011, Kulaw 2012). My research on red snapper reproduction at natural habitats will supplement the information available from artificial habitats, thus allowing me to examine the influence of habitat on red snapper reproduction.

Reproduction is a crucial part of fish stock recovery. A species like red snapper cannot be dependent on small, young females because maximum fecundity is achieved late in life (8 to 12 years) (Collins et al. 1996, Patterson et al. 2001, Woods 2003, Fitzhugh et al. 2004). Without a healthy spawning population of highly fecund females, the red snapper stock may never again reach its maximum reproductive potential (Trippel 1995). If there is a disparity in red snapper reproduction between natural and artificial habitats, those on the habitat type that is contributing more heavily may need to be managed more conservatively. Given these potential consequences it is surprising that natural habitats have received relatively little study.

STUDY OUTLINE

The second chapter of this thesis provides baseline information about red snapper reproduction at sites that have been little studied in the past. As such, this new study is an extension of a previous study that focused on the influence of habitat on red snapper ecology. We used red snapper biology (age, growth, diet analysis, nutritional analysis, and reproductive potential) combined with community structure and abundance (analysis using cameras and hydroacoustics) to examine the effects of habitat on populations of red snapper.

The objective of the third chapter of this thesis is to analyze red snapper reproduction at the same sites as chapter two, but grouped by habitat. I added an additional artificial habitat site for this part of the study. These are the same red snapper used in the larger collective study, only split into natural and artificial habitats and reanalyzed with the addition of the extra artificial site. Once again, my focus is on the reproductive aspects of the red snapper populations.

Both chapters analyze classic reproductive characteristics of fishes: GSI, weight-length relationships, size and age at maturity, batch fecundity, spawning frequency, and annual fecundity. Each of these characteristics were analyzed and then compared by site and habitat. Lastly, the potential impacts and implications of the comparisons are discussed.

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CHAPTER 2: SITE-SPECIFIC REPRODUCTIVE POTENTIAL OF RED SNAPPER (*Lutjanus campechanus*) ON THE LOUISIANA OUTER CONTINENTAL SHELF

INTRODUCTION

Shepard (1937) originally surveyed the reefs of the Louisiana/Texas continental shelf edge and hypothesized that the reefs were created from salt deposits left during the formation of the Gulf of Mexico. Salt domes have since pushed up through the sea floor, or are close to pushing through the sea floor, exposing rock from deeper basaltic layers (Rezak et al. 1990). Shepard (1937) was also the first to describe the carbonate sediment on the caps of the domes as “fragments of porous limestone mixed with abundant shells”.

The reefs examined in this study are part of a series of banks that are “bathymetric highs” situated on the Louisiana/Texas outer continental shelf (Shepard 1937, Gardner and Beaudoin 2005). Each reef has a unique physiography, but they are physically more similar to each other than they are to the surrounding mud/sand substrate. The reefs display general east to west differences in physical characteristics, likely due to proximity of the Mississippi River (Gardner and Beaudoin 2005). The reefs are shallow enough that light can penetrate to the sea floor and most of the year the seawater has relatively low turbidity, especially at the reefs more westward of the Mississippi River. The sunlight and low turbidity permits growth of both soft and hard corals, as well as calcareous algae (Rezak et al. 1990). The combination of shallow, clear seawater overlaying hard substrate creates a unique environment in the northern Gulf of Mexico (NGOM).

The reefs support a variety of fish and coral species with clear zonation of habitat by depth (Rezak et al. 1990). The original source of tropical larvae on the reefs is

possibly from the Loop Current, although today most of the reefs are likely self-sustaining. The Loop Current collected pelagic larva of tropical species as it flowed across the Caribbean and into the Gulf through the Yucatan Strait and then deposited these larva on the reefs (Thurman 2004). Subsequently, many of the reefs I studied exhibit high biodiversity compared to the adjacent mud and artificial habitats.

Temperature and light limits growth of hard corals, but soft corals and coral whips are abundant on the reefs. The age of the reefs (~15,000 years) and the high diversity of plants and animals have lead many to believe that this system of reefs constitutes the original center of abundance in the NGOM for reef associated species, such as red snapper (*Lutjanus campechanus*) (Goodyear 1995, Patterson et al. 2001). However, the vast majority of research on red snapper reproduction has been conducted from specimens collected on artificial habitats. The goal of my research was to extend the knowledge of red snapper reproduction to populations residing on natural habitats, specifically the shelf edge reefs (reefs) on the Louisiana continental shelf.

Red snapper support an economically important fishery in the NGOM. Sustainable harvest limits are set from stock assessments. Stock assessments rely, in part, upon the estimation of spawning stock biomass (SSB). SSB is weight of all the sexually mature female red snapper in the NGOM (Trippel 1995). Currently, the majority of information about red snapper SSB is from studies on artificial reefs (Appendix, Table A1). If red snapper resident at the shelf edge reefs are contributing to the NGOM population differently than those at artificial reefs, it will change the population dynamics of the stock. It is know that there are demographic differences in reproductive potential of red snapper east and west of the Mississippi River as well as regional differences

throughout the NGOM (Woods 2003, Jackson et al. 2007, Kulaw 2012, Sluis et al. 2012). This research is designed to define baseline data of red snapper reproductive potential at the natural reef habitats of the NGOM, as well as, gain a better understanding of a habitat within a demographic of the NGOM not yet fully understood.

Recently, differences in red snapper age structure, growth, and reproductive potential have been found between proximate artificial and natural habitats (Saari 2011, Kulaw 2012). It is known that red snappers tend to be associated with habitat containing vertical relief (natural and artificial) for an extended portion of their life cycle (Patterson et al. 2001, Workman et al. 2002). It is not surprising then that the original placement of oil and gas platforms caused a redistribution of adult red snapper on the Louisiana shelf. This shift in distribution may have influenced the demographics of the species in the NGOM. This project seeks to enhance the knowledge of populations currently resident at the Louisiana/Texas shelf edge reefs, specifically focusing on reproductive potential, because the majority of the information currently available is from artificial habitats.

METHODS

Sites

Red snapper populations at three sites on the Louisiana/Texas continental shelf edge were sampled: Jakkula Reef, McGrail Reef, and Bright Reef. Red snapper at one artificial reef site situated on a bathymetric high comprised of lithified delta muds in the East Cameron Artificial Reef Planning Area were also sampled (Figure 2.1) (Cowan et al. 2007). All four sites are located 130-200 km southwest of Port Fourchon, Louisiana.

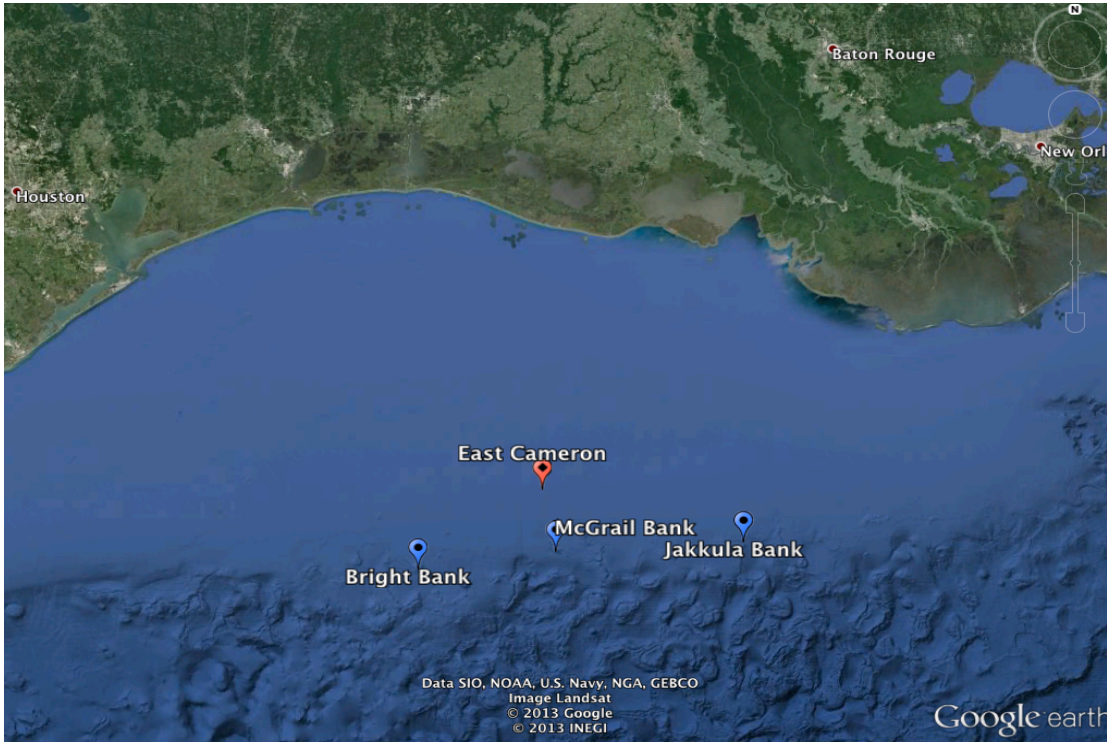


Figure 2.1 Collection sites for red snapper (*Lutjanus campechanus*). Jakkula Reef, McGrail Reef, and Bright Reef are naturally occurring shelf edge reefs; East Cameron is an artificial reef planning area that includes both standing and topples platforms. East Cameron is situated on a large patch of lithified delta mud (Cowan et al. 2007).

Jakkula Reef, the most easterly site and was sampled in an earlier study (Saari 2011, Kulaw 2012). The substrate at Jakkula Reef is composed mostly of sand and some mud with coral whips and rocky outcrops. Jakkula Reef is exposed to more sedimentation, relative to other natural sites in this study, because of its proximity to the mouth of the Mississippi River. Jakkula Reef also is the deepest of the sites, although it has the highest relief* at 92 m. The crest of the reef is the deepest of those in the study and it has the least amount of exposed hard substrate (Table 2.1) (Gardner and Beaudoin 2005).

*Relief is defined here as the vertical distance from the peaks of the summit to the adjacent seafloor (Gardner and Beaudoin 2005)

McGrail Reef is the smallest of the natural sites and has the least relief at 65m (Table 2.1). Two steep pinnacles separated by a deep valley distinguish McGrail Reef's profile. This site has the most stony coral of the sites we sampled and supports a diverse community of reef and pelagic fishes (Gardner and Beaudoin 2005). The substrate in this area is composed of many large rocky outcroppings. Near the pinnacles, the sea floor is covered in loose carbonate pebble-sized debris composed of coral rubble and shells.

Table 2.1 Physical characters of sites sampled. East Cameron is an artificial habitat, whereas, Jakkula, McGrail, and Bright are natural habitats. Values for East Cameron were calculated from acoustic data. Values for Jakkula, McGrail, and Bright were taken from Gardner and Beaudoin (2005), who define relief as the height from the top of the structure to the adjacent seafloor.

Site	Deepest point/shallowest depth (m)	Relief (m)	Dimensions (m ²)
Jakkula Reef	160/64	92	2890
McGrail Reef	110/45	65	13200
Bright Reef	130/33	75	29760
East Cameron	55/29	26	2580

Bright Reef is the most westerly site (Figure 2.1). The substrate is composed mostly of coral and calcareous algae rubble. Bright Reef is the largest and shallowest site with 75m of relief (Table 2.1) (Gardner and Beaudoin 2005) and is characterized by large rocky outcroppings that are well suited as reef-fish habitat. Hard corals are found on Bright Reef, but not in the abundance seen at McGrail Reef (Rezак et al. 1990).

The East Cameron site is situated within the East Cameron Oil and Gas Lease Area and is part of Louisiana Artificial Reef Program (LARP) that was founded in 1986 (Figure 2.1) (Kasprzak and Perret 1996). Since the start of the LARP, decommissioned oil and gas platforms have been moved to East Cameron and deployed as artificial reefs. The artificial habitat at East Cameron consists of both toppled and standing platforms. It has been shown previously that there are no significant differences in red snapper

reproductive potential at standing and toppled platforms (Kulaw 2012). East Cameron's artificial habitat is over twenty years old and is considered mature (Kasprzak and Perret 1996). A previous study described a two-kilometer ridge rising approximately two meters above the adjacent substrate at East Cameron; this is thought to be a patch of lithified delta mud (Cowan et al. 2007).

Sample Collection

Thirty hook or ten hook vertical longlines with alternating large and small hooks were used to harvest red snappers for this research. Hooks were spaced roughly one foot apart with enough weight (approximately 4-5 kg) at the terminus to keep the hooks vertical in the water column. Four longlines were deployed for one to three hours per site per trip, depending upon weather and sea condition. Single hook rods and reels were used either in conjunction with or instead of the longlines when appropriate. These methods caught a variety of species, although only red snapper were considered in this study.

All fish caught were fitted with a numbered tag and held on ice until dissection. All red snapper were measured for total length (TL, mm), fork length (FL, mm), standard length (SL, mm), total weight (TW, kg), and eviscerated body weight (EW, kg). Muscle tissue, fin clips, livers, stomachs, otoliths, and ovaries were removed at sea, frozen, and transported to either the Fisheries Science Laboratory or the Fish Age and Growth Laboratory at Louisiana State University (LSU).

Tissue Preparation

In the laboratory ovaries were thawed, blotted dry with paper towels, and weighed to the nearest 0.01 gram. The ovaries then were preserved in 10% formalin for no less than two weeks (longer if ovaries were very large). Large ovaries were punctured with a scalpel to allow for formalin to perfuse into and fix the inner ovary before bacteria could affect the oocytes (Nieland and Wilson 1995). Red snapper ovarian lobes are developmentally symmetrical; one sample from each ovary was sufficient for histological slide preparation and characterization of the reproductive state (Collins et al. 1996). Histological slides were then prepared from an ovarian cross section approximately 2 mm thick selected and prepared as follows. The ovary was visually divided into six sections; one section was chosen for sample extraction at random with a six-sided die (Appendix, A1). Cross sections were then placed in numbered histological cassettes. Fifty-six cross-sections were stored in 10% formalin solution and transported to the LSU School of Veterinary Medicine for slide preparation. The remainder of the histological slides were processed and made in the LSU Fish Age, Growth and Histology laboratory.

Slide Processing

Ovarian tissues were processed in a Leica ASP6025 tissue processor and then embedded in paraffin wax using a Leica EG 1150H embedding station. Tissues were then cut to 4 μ m with a Leica RM2125 RTS microtome. Cut tissues were attached to clean microscope slides in a warm water bath and transferred to a hot plate to dry. Then the slides were stained and counterstained with hematoxylin and eosin, respectively, in a Leica ST5020 Multistainer; a Leica CV5030 Coverslipper then applied cover slips.

Oocyte Stage Analysis

All slides from peak spawning season (June, July, and August) were examined for maturity with an Olympus BX41 microscope at 40x magnification. Oocytes were classified according to the four stages of oocyte development given by Wallace and Selman (1981) and Brown-Peterson et al. (2011): primary growth, cortical alveoli, vitellogenesis, and hydrated (Table 2.2, Appendix, A2-A4).

Based upon the designations developed by Wallace and Selman (1981) red snappers are heterochronal spawners with different stages of oocytes simultaneously occurring within the ovary during the spawning season. The presence of vitellogenic oocytes during spawning season was the benchmark for maturity in female red snappers (Nieland and Wilson 1993, Woods 2003, Jackson et al. 2005, McMillan 2007, Brown-Peterson et al. 2011).

Table 2.2 Biological explanation and histological characteristics of the progression of oocyte maturation for female heterochronal and asynchronous batch spawning marine teleosts. (Adapted from: Wallace and Selman 1981, Woods 2003, McMillan 2007, Brown-Peterson et al. 2011, and Kulaw 2012)

Phase	Biology	Histological Characters
Primary Growth (PG)	Centrally located large basophilic nucleolus; surrounded by squamous scale-like follicle cells; presence of only PG oocytes during the spawning season is indicative of immaturity or regenerative stage (Appendix, A2)	Oocytes small 20-200 μm ; little space between oocytes; oocytes dark purple in color with lighter core
Cortical Alveoli (CA)	The release of gonadotropin initiates the formation of “yolk vesicles”; small lipid droplets form around the nucleolus; CA oocytes are present immediately before vitellogenesis, but do not explicitly imply spawning capability (Appendix, A3)	Oocytes medium 200-500 μm ; purple in color, but lighter than PG; light core; lipids dissolve during preservation, leaving light ring of vesicles around nucleus

(Table 2.2 continued)

Phase	Biology	Histological Characters
Cortical Alveoli (CA)	The release of gonadotropin initiates the formation of “yolk vesicles”; small lipid droplets form around the nucleolus; CA oocytes are present immediately before vitellogenesis, but do not explicitly imply spawning capability (Appendix, A3)	Oocytes medium 200-500 μm ; purple in color, but lighter than PG; light core; lipids dissolve during preservation, leaving light ring of vesicles around nucleus
Vitellogenic (V)	Vitellogenin is released by the liver and transported to the ovaries via the bloodstream where it is cleaved into yolk proteins; the yolk proteins enter the oocytes by passing between the follicle cells, creating “lipid yolk droplets”; nucleus moves toward the animal pole; late V or early hydration appears homogeneous due to yolk coalescence; presence of V oocytes clearly reveals maturity (Appendix, A3)	Oocytes medium to large 0.50-1 mm; light purple/pink; distinctly lighter than CA or PG oocytes; grainy in appearance; appears homogeneous just before hydration
Hydrated (H)	Rapid increase in size due to water uptake- in morning for red snapper- immediately before spawning; homogeneous cytoplasm; formation of yolk plate; nucleus becomes difficult to identify; H oocytes indicate imminent spawning (Appendix, A4)	Largest oocyte size 1-2 mm; light pink; ‘squished balloon’ appearance due to dehydration during fixation; no nucleus visible
Postovulatory Follicles (POF)	Follicle walls collapse into empty space previously occupied by H oocyte creating network of distinct folds; red snapper POF degenerate within 24 hours; clearly folded POF indicate a female that has very recently spawned (Appendix, A5)	Small; light red/pink; consists of many collapsed folds
Atresia (A)	Oocyte is reabsorbed by phagocytosis; follicle layer becomes fragmented and the oocyte disintegrates; can be present in any stage but commonly occurs at the end of spawning season; also can be linked to nutritional condition (Appendix, A6)	Any size; discolored; dissolution of cell wall; oocyte still recognizable but clear break down at edges

Maturity was not assessed outside the spawning season because only primary growth and cortical alveoli stages are present, regardless of maturity. Maturity was also established by the presence of hydrated oocytes, postovulatory follicles (POF), or atretic oocytes within spawning season (Appendix, A4-A6). Ovaries with hydrated oocytes underwent further analysis to determine batch fecundity estimates, spawning frequency, and annual fecundity.

POF in ovaries indicate recent spawning within the previous 24 hours (Table 2.2) (Hunter and Macewicz 1985a, McMillan 2007). A POF is a layer of epithelial granulosa cells plus an outer layer of connective thecal tissue left from a hydrated oocyte after ovulation and spawning (Woods 2003). A POF is identifiable as a structure of collapsed folds that degrades quickly (within 24 hours).

Oocytes that are mature but not spawned usually are reabsorbed through a process called atresia. Atresia may be present at all stages of spawning, though it occurs more commonly at the cessation of spawning season (Hunter and Macewicz 1985b, Brown-Peterson et al. 2011). Because atresia is not present in immature fish, it can be an indicator of maturity, as well as the end of an individual's spawning season.

Gonadosomatic Index

Gonadosomatic indices (GSI) were calculated for all female red snapper sampled. GSI is a ratio of ovary mass to eviscerated body mass:

$$GSI = \frac{Ovary\ Mass\ (g)}{Eviscerated\ Body\ Mass\ (g)} \times 100$$

Increases in GSI indicate spawning readiness and potential investment of energy in

reproduction (Davies 1956, Collins et al. 1996). GSI values greater than one denote onset of spawning and peak spawning season in red snapper (Collins et al. 1996).

Size and Age at Maturity

Reproductively active female red snapper were sorted into both 50 mm TL size classes and age groups. The individuals in each class were then analyzed for 50% and 100% maturity.

Batch Fecundity Estimate

Those red snapper ovaries with hydrated oocytes, based upon histological evidence, were further examined to estimate batch fecundity. Batch fecundity (BF) is estimated gravimetrically from the count of the number of hydrated oocytes in a known weight of a subsample of ovarian tissue (Hunter et al. 1985). That number is then extrapolated to the weight of the entire ovary to obtain the approximate number of eggs that female would have spawned during the next spawning event, as follows:

$$BF = \left(\frac{\# \text{ hydrated oocytes}}{\text{sample wt (g)}} \right) * \text{whole ovarie wt (g)}$$

Three 0.03-0.05 g subsamples were taken randomly from among the six regions of the ovaries and placed on a microscope slide in a 3:7 glycerin-water solution. Hydrated oocytes were then visually counted under a compound microscope (Olympus BX41, 10x magnification)(Render and Wilson 1992, Wilson and Nieland 1994, Collins et al. 1996). The three BF estimates were averaged for each individual female.

Spawning Frequency

Red snapper are indeterminate batch spawners, periodically releasing batches of eggs over the course of a spawning season. In reproductively mature female ovaries, immature oocytes are continually developing into batches of mature oocytes. Each histological slide analyzed is only a snapshot of that female's reproductive season; this makes efforts more difficult when trying to estimate annual fecundity. This is why spawning frequency is used to estimate mature oocyte production.

Spawning frequency (SF) estimates the average number of days between consecutive spawning events or the number of days for all females in the population to spawn one time (Hunter and Macewicz 1985a, Nieland et al. 2002). There are three different methods for determining SF: the POF method, the hydrated oocytes method, and the time-calibrated method (Hunter and Macewicz 1985a, Fitzhugh et al. 1993, Wilson and Nieland 1994, Nieland et al. 2002, Woods 2003, Kulaw 2012). Each method has its own assumptions, but firstly it assumes that red snapper are indeterminate batch spawners. Methods for determining SF are as follows:

- 1) The POF method uses the total number of females with evidence of POF (day 1). After a fish spawns POF can only be recognized clearly within 24 hours post-ovulation (Table 2.2) (Wilson and Nieland 1994, Nieland et al. 2002). The spawning fraction is the proportion of females currently spawning on any given day. The inverse of the proportion of females with POF, or spawning fraction, represents the average number of days between spawning events for the population during spawning season. This method is difficult to use with a small data set; however, it is preferable to the hydrated method in most cases.

$$Spawning\ Fraction_{POF\ Method} = total\ \# \ day\ 1 / total\ \# \ mature$$

$$SF_{POF\ Method} = 1 / Spawning\ Fraction_{POF\ Method}$$

$$= total\ \# \ mature / total\ \# \ day\ 1$$

2) The hydrated oocyte method uses the ratio of the number of females with hydrated oocytes (day 0) to the total number of mature females in the sample population (Table 2.2) (Hunter and Macewicz 1985a). Spawning fraction is calculated in the same way as the POF method. The inverse of the proportion of females with hydrated oocytes or spawning fraction represents the average number of days between spawning events for the population during spawning season. The hydrated oocyte method assumes that all hydrated oocytes will be released as eggs within the next few hours. This method is not preferred because of potential sampling bias; females with hydrated oocytes can only be found during a short period of the day.

$$Spawning\ Fraction_{H\ Method} = total\ \# \ day\ 0 / total\ \# \ mature$$

$$SF_{H\ Method} = 1 / Spawning\ Fraction_{H\ Method} = total\ \# \ mature / total\ \# \ day\ 0$$

3) The TC or time-calibrated method takes into account both the numbers of females with either hydrated oocytes or POF. The TC method assumes ovaries that show oocytes with either yolk coalescence or hydration (day 0) will progress to spawning within the next 10-12 hours (Nieland et al. 2002). This method must

take into account the assumptions for both the hydrated and POF methods. The mean of the day 0 and day 1 females is used to calculate spawning fraction, instead of just one or the other. The inverse of the proportion of spawning fraction represents the number of days between spawning events for the population during spawning season. I used the TC method because it uses the greatest amount of data and will provide the most accurate SF, especially when sample sizes are small.

$$\begin{aligned}
 \text{Spawning Fraction}_{TC \text{ Method}} &= ((\text{total \# day 0} + \text{total \# day 1})/2) / \text{total \# mature} \\
 SF_{TC \text{ method}} &= 1 / \text{Spawning Fraction}_{TC \text{ Method}} \\
 &= \text{total \# mature} / ((\text{total \# day 0} + \text{total \# day 1})/2)
 \end{aligned}$$

To estimate the number of days for all females in the population to spawn one time I used a 150-day spawning season (Woods 2003).

$$\text{Spawning events per season} = \frac{150 \text{ days}}{SF}$$

Annual Fecundity

Annual fecundity (AF) is the estimate of the number of eggs a female red snapper releases during an entire spawning season. AF is calculated from the SF, a 150-day spawning season, and BF (Nieland and Wilson 1993, Woods 2003).

$$AF = \left(150 \text{ days} / SF \right) \times BF$$

AF was calculated for each female for which a BF was estimated.

Statistical Analysis

All statistical tests were done with the Statistical Analysis System (SAS v 9.3, Cary NC); statistical significance was determined at $\alpha=0.05$ for all tests. Analysis of variance (ANOVA) was used to compare sex ratios of red snappers between sites. Multivariate analysis of variance (MANOVA) was used to compare red snapper demographic characteristics by site. The demographic characteristics compared included: mean age, percent maturity, GSI, mean TL, mean TW, and seasonal bottom water temperature. Linear regression was used to calculate red snapper weight-length relationships and analysis of covariance (ANCOVA) was used to compare those relationships between sites. Linear regression was also applied to compare number of spawning events per season to TL and age. A Poisson regression was used to compare low numbers of red snapper batch fecundity estimates between the East Cameron site and the other sites having red snapper with batch fecundity estimates.

RESULTS

A total of 523 red snappers were collected from the East Cameron Artificial Reef Planning Area (East Cameron), Jakkula Reef, McGrail Reef, and Bright Reef from 2011 to 2013. Of these, 273 (52%) were collected during peak spawning months (June, July, and August) and 121 (44%) were females. The ratio of females was over 50% at both Jakkula Reef and McGrail Reef, but not at Bright Reef or East Cameron (Table 2.3).

Table 2.3 Sex ratios of red snapper (*Lutjanus campechanus*) at all sites sampled during peak spawning months (June July, August). Superscripts^{wxyz} show differences at $p < .05$ between individual sites, determined with an ANOVA.

Site	Female	Male	% Female
Jakkula Reef ^w	22	7	78% ^{yz}
McGrail Reef ^x	9	7	56% ^y
Bright Reef ^y	32	72	31% ^{wxz}
East Cameron ^z	58	66	47% ^{wy}

Red snapper in my samples ranged from 3-17 years (M. Kormanec, This study); the mean age for individual at 3 years and Bright Reef had the oldest fish at 17 years (Table 2.4). TW ranged from 0.45 to 7.65 kg; the mean TW for all individuals is 2.28 ± 1.41 kg. East Cameron on average had the leanest red snapper while Jakkula Reef had the heaviest (Table 2.4). TL ranged from 327 to 793 mm with a mean of 540 ± 115 mm; East Cameron had the smallest individuals while Jakkula Reef had the largest (Table 2.4). Multivariate analysis of variance (MANOVA)

Table 2.4 Demographic characteristics of female red snapper (*Lutjanus campechanus*) sampled at individual sites during spawning season (June, July, August). Least squares means \pm standard error or N (%). Superscripts^{abcd} show differences at $p < .05$ between individual sites, determined with a MANOVA.

Characteristic	Jakkula Reef ^a	McGrail Reef ^b	Bright Reef ^c	East Cameron ^d
N (females) †	22	9	32	58
Age (years)	8 ± 1 ^{bd}	6 ± 1 ^a	7 ± 1 ^d	5 ± 1 ^{ac}
Age Range (years) †	6-12	4-10	4-17	3-9
Number and % Mature (females only)	22 (100%) ^d	9 (100%) ^d	31 (97%) ^d	25 (44%) ^{abc}

(Table 2.4 continued)

Characteristic	Jakkula Reef ^a	McGrail Reef ^b	Bright Reef ^c	East Cameron ^d
Gonadosomatic Indices	1.85 ± 0.21^{bd}	2.69 ± 0.32^{acd}	1.65 ± 0.17^{bd}	0.57 ± 0.13^{abc}
Total Length (mm)	647 ± 22^{cd}	638 ± 33^{cd}	524 ± 18^{ab}	498 ± 13^{ab}
Total Weight (kg)	3.76 ± 0.27^{cd}	3.39 ± 0.40^{cd}	1.86 ± 0.21^{ab}	1.86 ± 0.16^{ab}
Seasonal Bottom Temperature (°C)	22 ± 1^{cd}	23 ± 1^d	23 ± 1^{ad}	25 ± 2^{abc}

† Not included in a statistical model-used only to show trend

showed differences in red snapper mean age, mean TW, and mean TL among sites (Table 2.4). Specimens from East Cameron and Bright Reef had significantly lower mean TW and TL than those from Jakkula Reef and McGrail Reef.

Length-weight analysis of red snapper TL and eviscerated body weight (EW, kg) was done on all female red snapper caught during peak spawning months. A predictive regression fitting TL to standard lengths (SL, mm) was used to estimate missing TL ($r^2 = 0.8816$, slope = 1.15, intercept = 28.09). EW and TL from all sites were \log_{10} transformed and EW was regressed on TL. All regressions were significant ($P < 0.0001$) and all regression coefficients were well correlated ($r^2 > .90$)(Bright, slope = 2.916, intercept = -4.71)(Jakkula, slope = 3.16, intercept = -5.36)(McGrail, slope = 3.10, intercept = -5.22)(East Cameron, slope = 2.92, intercept = -4.68) (Figure 2.2). Length-weight relationships for red snapper were tested between sites with an analysis of covariance (ANCOVA). The ANCOVA showed that all sites have slopes that are

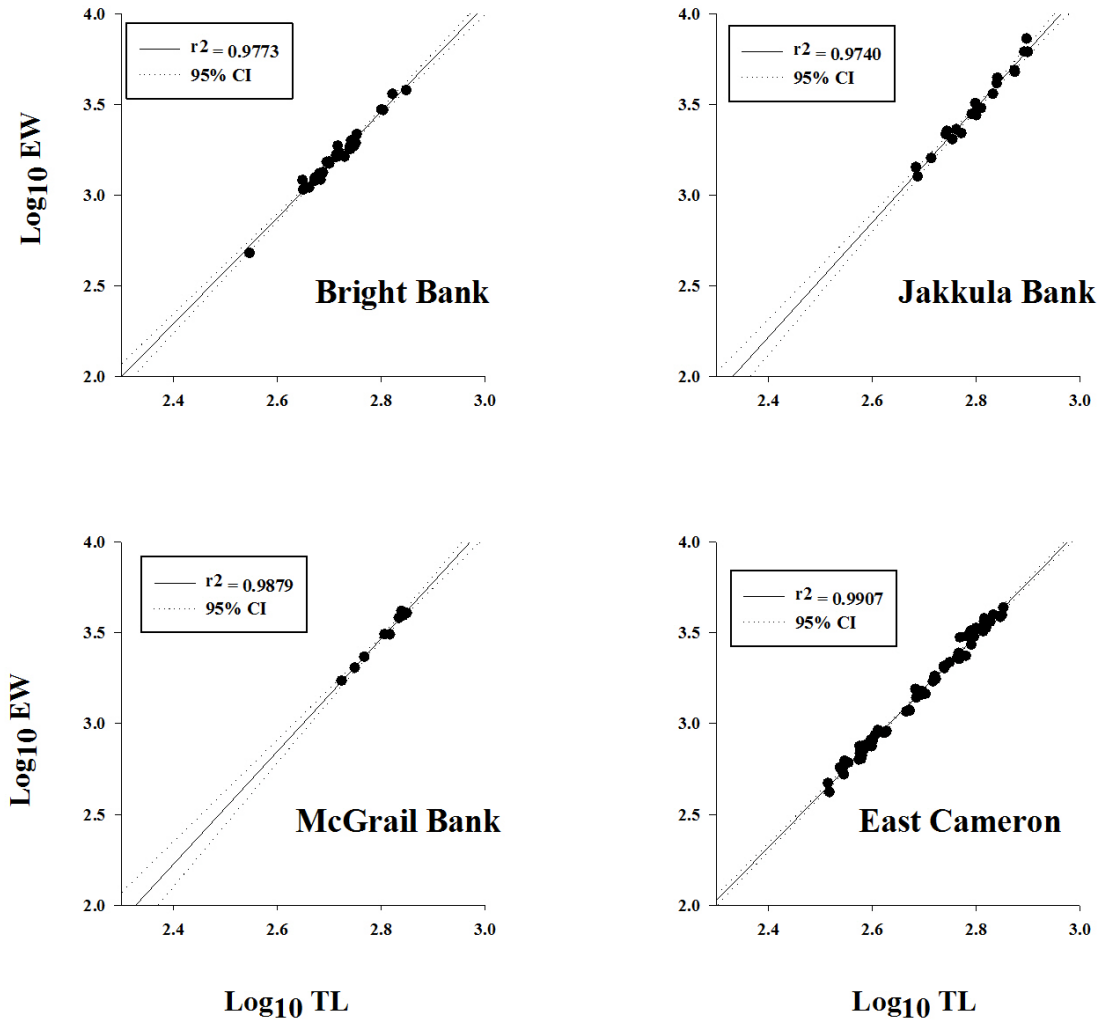


Figure 2.2 Length-weight regressions for female red snapper (*Lutjanus campechanus*) sampled at each site during peak spawning months (June, July, and August). EW = eviscerated body weight (g); TL = Total length (mm).

significantly different from zero ($p < 0.0001$) as are the intercepts ($p = 0.0003$). When slopes were compared between sites, East Cameron is significantly different from Jakkula Reef ($p = 0.0046$) and Bright Reef ($p = 0.0001$), while being not significantly different from McGrail Reef ($p = 0.0772$). There are no statistically significant differences in slopes between the reefs (Bright and Jakkula $p = 0.7653$; Bright and McGrail $p = 0.6731$; McGrail and Jakkula $p = 0.8097$).

Reproductive Analysis

Gonadosomatic Index

The mean gonadosomatic index (GSI) of all female red snapper caught during peak spawning months was 1.23 ± 1.16 with a range of 0.14 to 7.59. East Cameron red snapper had the lowest mean GSI while McGrail Reef red snapper had the highest (Table 2.5).

Red snapper GSI has been reported to rise in May then peak June until August and decline in September (Grimes 1987, Collins et al. 1996). McGrail Reef and East Cameron red snapper had significantly different mean GSI compared to the other two

Table 2.5 Mean, minimum (Min), maximum (Max), standard deviation (StDev.) and 95% confidence intervals (95% CI) for gonadosomatic indices (GSI) for female red snapper (*Lutjanus campechanus*) caught during spawning season (June, July, August) of 2011-2013.

Site	N	Mean	Min	Max	StDev.	95% CI
Jakkula Reef	22	1.78	0.35	7.59	1.54	(1.10, 2.46)
McGrail Reef	9	2.69	0.80	4.98	0.71	(1.81, 3.47)
Bright Reef	32	1.65	0.21	3.82	0.85	(1.34, 1.96)
East Cameron	58	0.57	0.14	3.51	0.62	(0.41, 0.73)

sites during peak spawning months (Table 2.4). The trend shown in Figure 2.3 indicates that red snapper at the three reef sites have higher mean monthly GSI during peak spawning months. Figure 2.3 also shows that red snapper GSI during the non-spawning months (October thru April) is low (<1) for all sites.

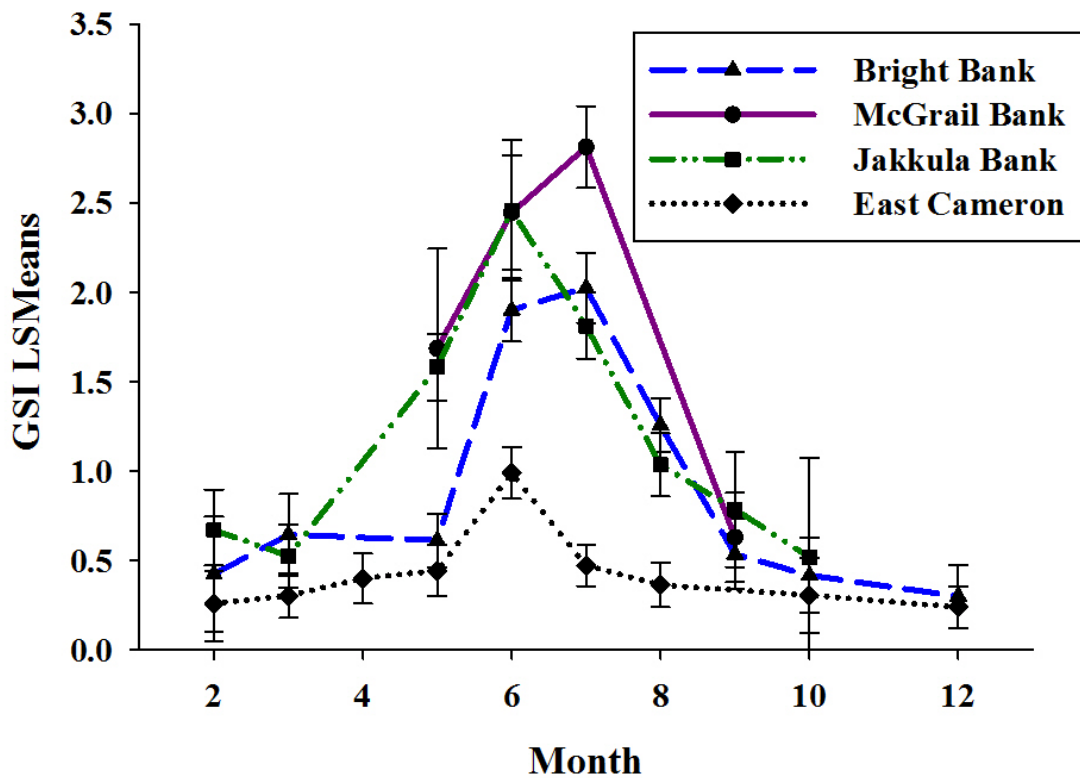


Figure 2.3 Mean monthly gonadosomatic indices (GSI) for female red snapper (*Lutjanus campechanus*) at all sites; verticle bars represent standard errors of monthly means. Least squared means are used instead of actual means to give a better representation of compared values.

Maturity

The smallest specimen to achieve maturity (presence of vitellogenic oocytes) was 301 mm TL and 3 years old from East Cameron; McGrail and Bright Reefs both had mature 4-year-old individuals. McGrail Reef red snapper minimum TL at maturity was 529mm, while a Bright Reef specimen achieved maturity at 445mm. Results from McGrail Reef should be interpreted with caution because of low sample size of mature individuals. Jakkula Reef red snapper minimum age and TL at maturity was 6 years and 483mm, respectively.

Maturity-at-age and maturity-at-size was 100% for red snapper at all the reef sites, except for Bright Reef, which had one 352 mm, 6 year old individual that was not mature (Table 2.6 and 2.7). East Cameron had the smallest and youngest individual mature red snapper; 50% maturity-at-size was reached for the 351-400mm size class, although three of the next four size classes show less than 50% maturity (Table 2.6). Maturity-at-age for East Cameron red snapper was 50% at age 7 and 100% at age 8 (Table 2.7). East Cameron red snapper have a significantly lower overall maturity at age and TL than those at the three reef sites (MANOVA, Table 2.4).

Table 2.6 Percent maturity for red snapper (*Lutjanus campechanus*) within a total length size class. * Indicates 5 or fewer specimens for that group.

Total Length (mm)	Jakkula Reef	McGrail Reef	Bright Reef	East Cameron
301-350	-	-	-	0*
351-400	-	-	0*	54
401-450	-	-	100*	20*
451-500	100*	-	100	63
501-550	100*	100*	100	17
551-600	100*	100*	100	40*
601-650	100	100*	100*	50
651-700	100*	100*	100*	67
701-750	100*	100*	100*	67*
751+	100*	-	-	-

Table 2.7 Percent maturity for red snapper (*Lutjanus campechanus*) for specific age groups. * Indicates 5 or less individuals for that group.

Age (years)	Jakkula Reef	McGrail Reef	Bright Reef	East Cameron
3	-	-	-	25*
4	-	100*	100*	43
5	-	100*	100	38
6	100*	100*	80*	23
7	100*	-	100	83
8	100*	100*	100*	100*
9	100*	-	100*	100*
10+	100*	100*	100	-

Spawning Indicators

Only nine of the red snapper I collected had hydrated oocytes indicative of imminent spawning: seven from East Cameron, one from Jakkula Reef, one from Bright Reef, and none from McGrail Reef. The smallest individual with hydrated oocytes was 4 years old and 351 mm TL from East Cameron. The largest individual with hydrated oocytes was 10 years old and 789 mm TL from Jakkula Reef. Bright Reef had the highest percentage of red snapper ovaries with POF, indicative of recent spawning, present (28%) and East Cameron had the lowest (5%) despite having the largest sample size ($n = 58$) (Table 2.8).

Table 2.8 Descriptive fecundity characteristics for female red snapper (*Lutjanus campechanus*) sampled at individual sites during spawning season (June, July, August). Means \pm SD or N (%). *Spawning events per season are based on a 150 day spawning season.

Characteristic	Jakkula Reef	McGrail Reef	Bright Reef	East Cameron
Hydrated Oocytes in Ovaries	1 (5%)	0	1 (3%)	7 (12%)
Postovulatory Follicles in Ovaries	5 (23%)	0	9 (28%)	3 (5%)
Batch Fecundity Estimate (eggs/batch)	1,194,993	NA	214,132	41,080 \pm 48,027
Annual Fecundity Estimates (eggs/season)	44,812,250	NA	7,834,180	1,467,143 \pm 1,715,271

Batch Fecundity

The overall mean batch fecundity BF for all hydrated specimens was 188,963 \pm 384,036 eggs per batch; the standard deviation is high because the range was 6,991 to 1,194,993 eggs per batch. Jakkula Reef red snapper had the highest BF estimate while those from East Cameron had the lowest (Table 2.8). Batch fecundity estimates for East

Cameron red snapper are generally low regardless of size or age when compared with estimates from the other two sites (Figure 2.4). The BF estimates for red snapper at East Cameron are all low, otherwise there are no discernable patterns. There are no BF estimates for McGrail because no specimens with hydrated oocytes were collected there. Due to the small sample size, red snapper mean BF could not be compared among all sites. However, a Poisson regression was used to show that there are differences in BF estimates of red snapper between East Cameron, Jakkula Reef, and Bright Reef. East

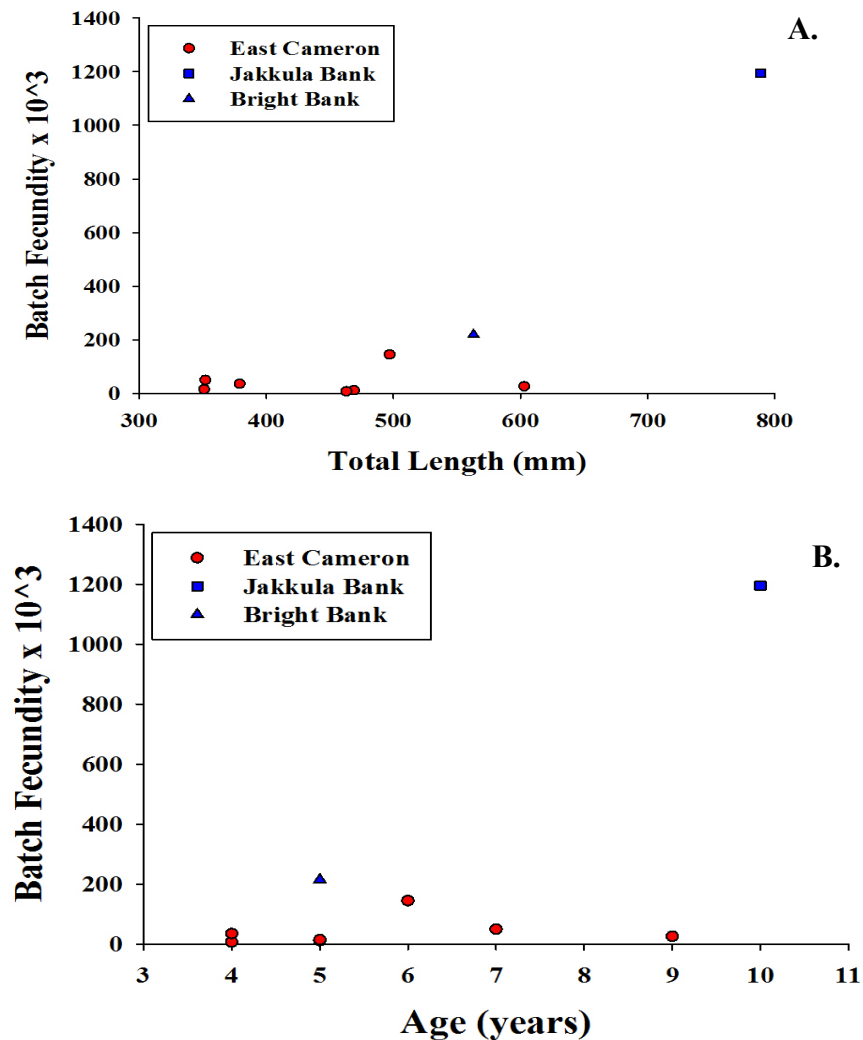


Figure 2.4 Batch fecundity of female red snapper (*Lutjanus campechanus*). A = total length (mm), B = Age (years)

Cameron BF data points were used to regress BF estimates on TL. Then only the TL values were added for red snapper females with hydrated oocytes from both Jakkula Reef and Bright Reef. This forced the model to predict the missing BF values based on the East Cameron values. The model turned out to be significant (ChiSq, $p < 0.0001$). The upper and lower bounds of the model prediction did not include the estimates of red snapper BF values from both Jakkula Reef and Bright Reef (Table 2.9); this means that if the BF values for Jakkula Reef and Bright Reef were added to the model they would be outliers. Because the BF values from Jakkula Reef and Bright Reef would be outliers, they are different from the East Cameron BF values.

Table 2.9 Shows results from Poisson Regression of batch fecundity estimates from female red snapper (*Lutjanus campechanus*) with ovaries containing hydrated oocytes. Estimated Value is the exact batch fecundity estimate measured from the specimens. The model predicted value and upper/lower bounds are the batch fecundities estimated by the regression model. Values are in eggs per batch.

Site	Estimated Value	Model Predicted Value	Upper bound of the Model	Lower Bound of the Model
Jakkula Reef	1,194,993	84,307	85,296	83,330
Bright Reef	214,132	46,196	46,378	46,015

Spawning Frequency

According to the POF method, female red snapper at East Cameron spawn 18 times per season, whereas red snapper at Jakkula Reef and Bright Reef spawn more often during the year (Table 2.10). No females with POF were found at McGrail Reef, therefore there are no POF method estimates for McGrail Reef. The spawning fraction for East Cameron is also lower than both Jakkula Reef and Bright Reef (Table 2.10).

According to the hydrated oocyte method females at McGrail Reef spawned every 1.5 days and have 100 spawning events per season (Table 2.10). This method over estimated values at McGrail Reef because of the nine mature females found at McGrail

Reef, six of them had late vitellogenic oocytes (Table 2.10). After McGrail Reef, spawning frequency established by the hydrated method was highest at East Cameron followed by Jakkula Reef and lastly Bright Reef (Table 2.10).

Based on the time calibrated method spawning was estimated to occur every four days for all sites except McGrail Reef (Table 2.10). Given a 150-day spawning season, 36 spawning events would have been expected at Bright Reef and East Cameron (Table 2.10). Spawning occurred more frequently at Jakkula Reef with 38 expected spawning events per season. The 50 expected spawning events for McGrail Reef is likely due to lack of females with POF.

Table 2.10 Spawning frequency calculation methods of female red snapper (*Lutjanus campechanus*) sampled during spawning season (June, July, August). Spawning events per season were calculated with a 150 day spawning season defined by Woods 2003.

	Jakkula Reef	McGrail Reef	Bright Reef	East Cameron
Mature Females	22	9	31	25
Postovulatory Follicles (day 1)	5	0	9	3
Ovaries with Hydrated Oocytes (day 0)	1	0	1	7
Ovaries with Late Vitellogenic Oocytes (day 0)	5	6	5	2
<u>POF Method</u>				
Spawning Fraction (estimated proportion of mature females spawning during any day in spawning season)	23%	0%	29%	12%
Spawning Frequency Estimate (days between spawning)	4.4	0	3.4	8.3
Spawning Events per Season	34	0	44	18

(Table 2.10 continued)

	Jakkula Reef	McGrail Reef	Bright Reef	East Cameron
<u>Hydrated Oocyte Method</u>				
Spawning Fraction (estimated proportion of mature females spawning during any day in spawning season)	27%	67%	19%	36%
Spawning Frequency Estimate (days between spawning)	3.7	1.5	5.2	2.8
Spawning Events per Season	41	100	29	54
<u>Time Calibrated Method</u>				
Spawning Fraction (estimated proportion of mature females spawning during any day in spawning season)	25%	33%	24%	24%
Spawning Frequency Estimate (days between spawning)	4.0	3.0	4.1	4.2
Spawning Events per Season	38	50	36	36

Spawning frequency of red snapper was estimated at age and TL for all sites combined due to low sample sizes at individual ages and TL per site. Larger individuals tended to spawn more often, except in the 350mm and 400mm groups (Figure 2.5). A simple linear regression showed the slope of TL and spawning events per season was not different from zero ($P = 0.3988$, $r^2 = 0.1034$). Older red snapper tended to spawn more often with the exception of age 4 fish (Figure 2.5). Age 4 red snapper having a spawning frequency of 50 events per season is most likely due to small sample size and is probably not biologically significant. The relationship between age and spawning events per season also had a slope not significantly different from zero ($p = 0.3731$, $r^2 = 0.1605$).

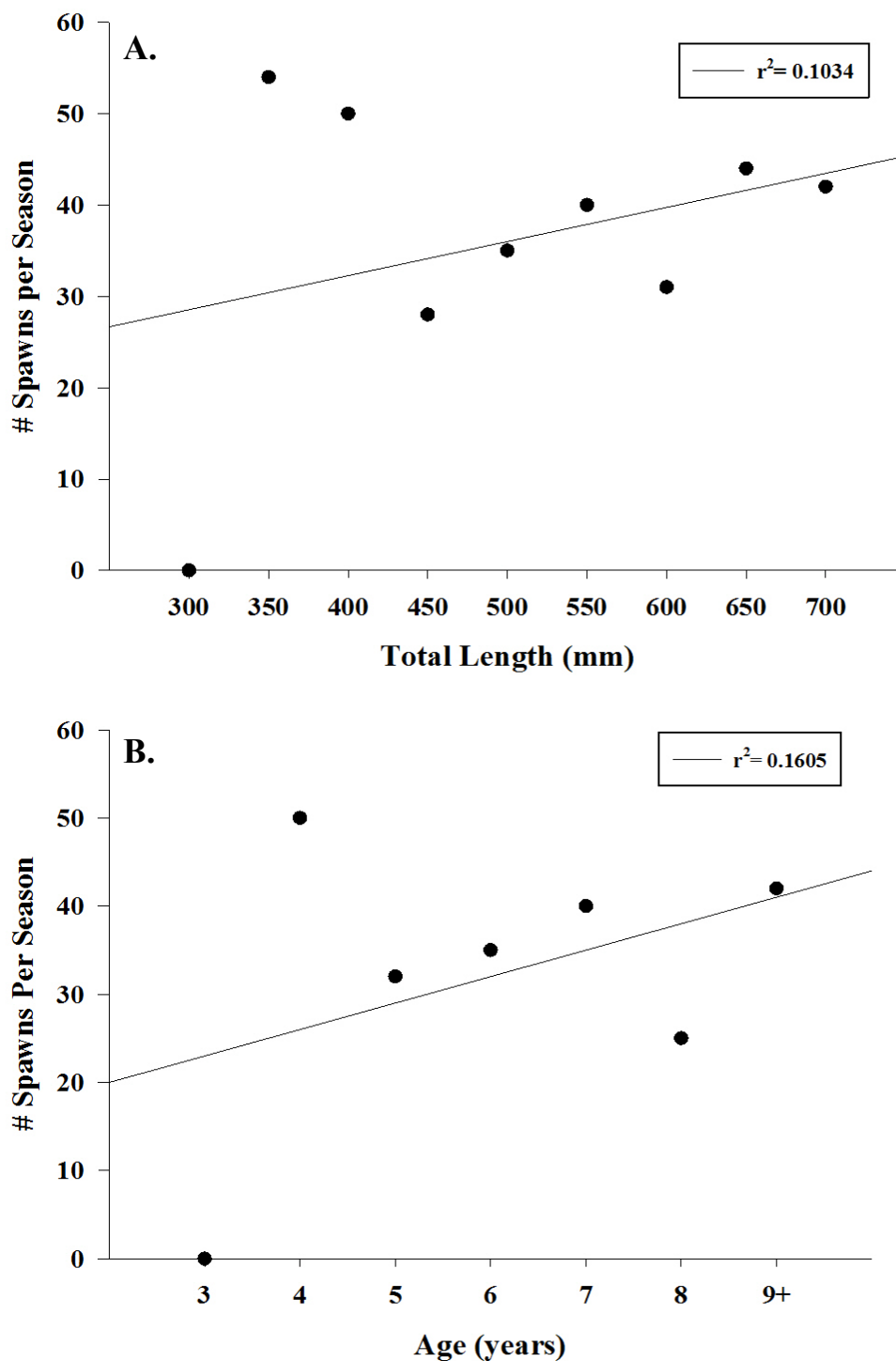


Figure 2.5 Spawning events per season for red snapper (*Lutjanus campechanus*), sampled from sites Jakkula Reef, McGrail Reef, Bright Reef, and East Cameron. Seasonal spawning events estimated with a 150-day spawning season and the time-calibrated method from Fitzhugh et al. 1993 and Nieland et al. 2002. Spawning events by A) total length (mm) and B) age (years). 9+ includes all fish above the age of 9.

Annual Fecundity

Annual fecundity for red snapper was calculated with the time-calibrated estimate for BF and with SF estimates; the mean for all sites combined was $6,990,707 \pm 14,415,278$. The large standard error is due to the range of the annual fecundity estimates between red snapper at East Cameron and those sampled from other sites. East Cameron red snapper had the lowest estimated annual fecundity and Jakkula Reef red snapper had the largest (Table 2.8). A larger data set would have allowed better estimates of annual fecundity.

DISCUSSION

Overall, this study shows that the reproductive potential of red snapper at East Cameron was significantly different from that at the three reef sites (Jakkula Reef, McGrail Reef, and Bright Reef) but the reef sites were not different from each other. The most likely mechanism for differences in reproductive potential between the four sites is dissimilar habitat types. Reproductive potential in teleost species is generally influenced by age, size, condition of individual fish, and condition of the stock as a whole (Marteinsdottir and Begg 2002). The East Cameron Artificial Reef Planning Area is situated on a patch of lithified delta mud, but the population of red snapper at the site inhabits an artificial reef. Jakkula Reef, McGrail Reef, and Bright Reef are habitats formed from natural processes and the red snapper population is directly associated with naturally formed substrate. The hard bottom system that connects Jakkula Reef, McGrail Reef, and Bright Reef is likely the historical center of abundance for red snapper in the NGOM and also support a species rich reef ecosystem (Rezak et al. 1990, Goodyear

1995). There is lower species diversity at East Cameron although there is high species abundance (Langland T., this study, Simonsen et al. 2014). These differences in habitats will affect the condition of female red snappers and condition will in turn affect reproductive potential.

This study suggests that the red snappers at East Cameron have different morphology than the red snappers at the reef sites and that red snapper at the reef sites have similar morphology to each other. A previous study reached a similar conclusion, finding that red snapper on Jakkula Reef were shorter but heavier-at-size than red snapper in adjacent artificial habitats (Saari 2011, Kulaw 2012). One study has linked short but heavy body size to early onset of maturity due to a surplus of somatic energy, allowing energy to be devoted to reproduction instead of somatic growth (Morgan 2004). Therefore, discrepancies in morphology could indicate differential energy allocations between somatic and gametic growth for red snappers at East Cameron and at the reef sites, thus leading to the reproductive differences observed between the two habitat types and the reproductive similarities among the three reef sites.

East Cameron also exhibited a shorter spawning season than those at the reef sites. Red snapper have a well-documented spawning season of May to September (Collins et al. 1996, Woods 2003, Kulaw 2012). Normally, spawning season is accompanied by $GSI > 1$ for red snappers, indicating females have enlarged ovaries relative to their body size and are spawning or are preparing to spawn (Davies 1956, Grimes 1987, Collins et al. 1996, Brown-Peterson et al. 2011). The female red snappers at East Cameron did not exhibit a mean $GSI > 1$ for the entirety of the spawning season, leading to the conclusion that the spawning season at East Cameron is truncated. There is

a possible bias because of lower sample sizes at the reef sites, but the results support a normal spawning season at the reef sites. A contracted spawning season is an indicator of a stressed population (Trippel 1995). Given the anomalous GSI observations, the red snappers at East Cameron may be exhibiting skip spawning, causing them to allocate energy away from reproduction and thus shortening the spawning season. A mature female that “chooses” not to spawn during spawning season is skip spawning. Skip spawning in adult fishes is not based on size or age, but is correlated to liver condition (Morgan and Lilly 2006, Rideout et al. 2006).

Stress hormones negatively affect liver condition, which affects the onset of reproduction. The liver produces a hormone that triggers oocytes to mature and stress causes a reallocation of energy, specifically away from reproduction (Schreck et al. 2001, Morgan 2004, Rideout et al. 2006, Schreck 2010). Poor diets and overt intraspecies competition can cause stress in fish populations (Thresher 1983, Rideout et al. 2006). Community studies concurrent during this project have shown that the population at East Cameron is dominated by red snapper and the populations at the reefs sites are more species diverse (Langland, personal correspondence). Furthermore, diet analysis of the red snapper in this study has shown that the diet at East Cameron is nutritionally poor when compared to the diets at the reef sites (Schwartzkopf 2014).

Female red snappers at East Cameron showed a slower progression to sexual maturation than those at the reef sites. Previous studies report 50% maturity-at-age of red snappers at 2-4 years (Woods et al. 2003, Jackson et al. 2007, Kulaw 2012); East Cameron red snappers achieved 50% maturity-at-age at 7 years and 100% at 8 years. Previous studies have reported 100% maturity of red snapper as early as 5 and 6 years old

(Woods 2003, Fitzhugh et al. 2004, Jackson et al. 2007, Kulaw 2012). The red snapper at the reef sites showed 100% maturity for all age groups and size classes represented, with the exception of one 6 year old from Bright Reef. When maturity-at-size data from this study contradict other studies of red snapper in the NGOM. The most recent study in this area showed 50% of red snapper at 450 mm TL are mature with 100% mature at 700 mm TL (Kulaw 2012). Earlier studies report 100% of red snapper are mature at 650 mm fork length (FL) (Woods 2003, Fitzhugh et al. 2004). 100% maturity at the three reef sites is first noted at 450 mm TL. The red snapper at the reef sites are maturing at younger ages and smaller sizes than red snapper at East Cameron.

Maturity in fish can be triggered at young ages and small sizes when the population is experiencing juvenescence or when energy thresholds are surpassed (Trippel 1995, Silverstein et al. 1997). Juvenescence is an unlikely mechanism for the early onset of maturity because it is a compensatory response normally caused by the removal of the larger older breeding stock (Trippel 1995). The red snappers at the three reef sites are maturing at younger ages and smaller sizes despite the presence of large older females. It is more likely that the red snappers at the reef sites have a surplus of energy and can allocate energy to reproduction instead of somatic growth (Silverstein et al. 1997). The red snappers at the reef sites are feeding on richer food sources and are experiencing less intraspecies competition (Langland T., personal correspondence) (Thresher 1983, Simonsen 2013, Schwartzkopf 2014, Simonsen et al. 2014). Therefore, the red snappers at the three reef sites are in better nutritional condition compared to those at East Cameron (Schwartzkopf 2014). The red snapper at the reef sites are maturing early because they are in better condition than the red snapper at East Cameron.

The most informative metric of reproductive potential is BF; however, in this only nine red snappers were obtained with hydrated oocytes in the ovaries. BF in red snappers conventionally increases with age and size (Collins et al. 1996). These data do not show a relationship between BF and age or TL, most likely due to a small sample size. However, the mean BF of red snapper at East Cameron is lower than mean BF estimates reported in other studies; whereas, the BF values from Jakkula Reef and Bright Reef align with previously reported mean BF estimates for red snapper (Collins et al. 1996, Woods 2003, Kulaw 2012). Reduced fecundity has been linked to poor diet and poor condition in fish (Marteinsdottir and Begg 2002, Rideout et al. 2006). Therefore, the lower BF at East Cameron may be attributed to the poor condition of red snapper there when compared to the red snapper at the three reef sites.

Spawning frequency of red snapper showed a slight increase with age and TL when data from all sites were combined, although the trend was not statistically significant. This does not agree with earlier studies. Normally SF increases with both age and TL (Woods 2003, Fitzhugh et al. 2012). The non-significant trend can most likely be attributed to the small sample size. The best estimates of SF are found when a population can be sampled over a 24-hour period during spawning season. For my analysis we had limited time at each site so I had to combine all the females caught over the peak spawning season. This could have biased the results. The individual site based estimates of SF agreed with previous studies of red snapper (Collins et al. 1996, Woods 2003, Kulaw 2012). These results show that despite differences in BF estimates, red snapper at all the sites are spawning the same number of times per season.

Annual fecundity (AF) estimates suggest that, while red snappers at each site are spawning at similar frequencies, they are releasing fewer eggs at East Cameron over the spawning season and potentially contributing less to the future population. The red snappers at East Cameron had relatively a low mean AF of 1.5 ± 1.7 million eggs per season per female with respect to other studies. Kulaw (2012) showed higher AF estimates of 3-7 million eggs per year per red snapper female. Earlier studies with larger sample sizes reported much higher AF estimates of 12 to 60 million eggs per year in Florida and 19 to 23 million eggs per year in Louisiana/Alabama (Collins et al. 1996, Woods 2003). Red snapper AF estimates from Jakkula Reef, 44.8 million eggs per season, and Bright Reef, 7.8 million eggs per season, correspond better with the previously reported values of AF estimates.

The evidence presented here suggests that red snappers reproduction at East Cameron contributes less to the entire NGOM population than the red snapper at Jakkula Reef, McGrail Reef, and Bright Reef. The red snappers at the three reef sites are in better condition than the red snapper at East Cameron and therefore appear to allocate more energy to reproduction (Marteinsdottir and Begg 2002, Morgan and Lilly 2006, Schwartzkopf 2014). This causes the red snapper at the reef sites to mature earlier, have higher GSI, and a normal spawning season. Red snappers at East Cameron consistently had low GSI, lower rates of maturity, and a shorter spawning season than at the three reef sites. The results of this study state that reproductive traits in female red snappers are consistent within a specific habitat type, but differ greatly between habitat types.

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CHAPTER 3: A COMPARISON OF RED SNAPPER (*Lutjanus campechanus*) REPRODUCTIVE POTENTIAL IN THE NORTHERN GULF OF MEXICO: NATURAL VERSUS ARTIFICIAL HABITATS

INTRODUCTION

The vast majority of research on adult red snapper has been conducted at artificial habitats; particularly oil and gas platforms. Previously studies have determined that there are differences in red snapper demographics in the Gulf of Mexico (Appendix, Table A1) (Woods et al. 2003, Jackson et al. 2007, Sluis et al. 2012). Artificial habitats on the Louisiana outer continental shelf consist primarily of standing and toppled oil and gas platforms; they have significant vertical relief, but relatively small area or footprint on the seafloor (Figure 3.1) (Gardner and Beaudoin 2005, Cowan et al. 2007). Most natural habitats on the Louisiana outer continental shelf consist of relief that gradually grades over a large area (Figure 3.1). While vertical relief characterizes both natural and artificial habitats in the northern Gulf of Mexico (NGOM), the relief is not structured the same at both habitats. Most natural habitats on the Louisiana outer continental shelf consist of relief that gradually grades over a large area, so the footprint is very large (Figure 3.1). The lack of attention that the natural reefs in the NGOM have garnered is surprising given that the reefs off Louisiana and Texas are believed to be the historical center of abundance for the species. I suspect this occurred as a matter of convenience because artificial habitats are easily located and closer to shore than most of the natural habitats in the NGOM. More recently, new studies off Louisiana have focused directly on the comparisons of red snapper demographics from artificial and natural habitats (Saari 2011, Kulaw 2012).

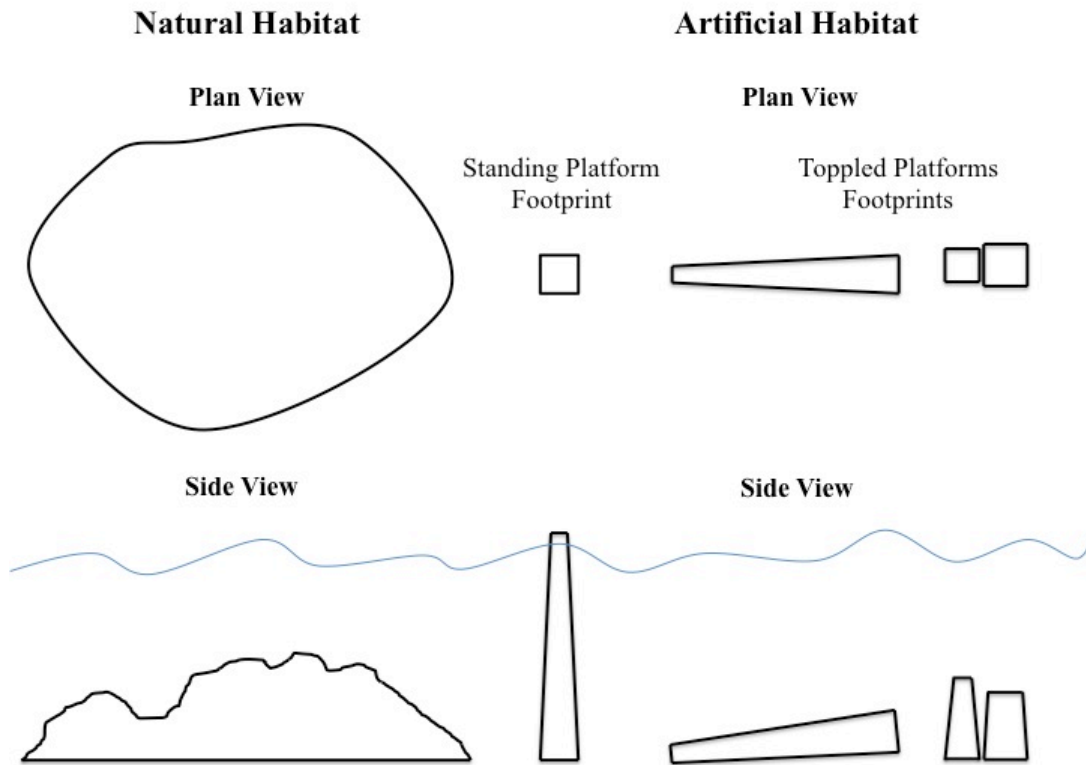


Figure 3.1 Illustration of the physical difference in habitat configuration between natural and artificial habitats. Plan view is the top-down or horizontal view. Side view is vertical view. Because of the varying shapes and sizes of both habitats, this figure is not drawn to scale.

It is known that red snapper tend to be associated with habitat having vertical relief (natural and artificial) for an extended portion of their life cycle (Patterson et al. 2001, Workman et al. 2002). It is not surprising, then, that the placement of thousands of oil and gas platforms caused a redistribution of adult red snapper on the Louisiana shelf. This shift in distribution also may have influenced the demographics of red snapper in the NGOM. If there is a significant difference in the reproductive potential of red snapper between natural versus artificial habitats, the relative contribution of new recruits may be dependent in part upon the proportion of females living on each habitat type.

The Gulf of Mexico currently contains more than 2,400 standing oil and gas platforms (Decomworld 2013). In 1986 the Louisiana Artificial Reef Program (LARP) was initiated to take advantage of the habitat created from obsolete platforms (Kasprzak and Perret 1996). LARP created nine Artificial Reef Planning Areas on the continental shelf of Louisiana where some decommissioned oil and gas platforms could be repurposed exclusively as artificial habitat. This created a cost effective way for oil and gas companies to retire platforms while contributing artificial habitat both for fishes and for fishing opportunities. Since the founding of LARP, over 4000 platforms have been removed while more than 300 have been moved to LARP planning areas (Kasprzak and Perret 1996, Decomworld 2013).

The LARP artificial habitats make up less than ten square kilometers of artificial hard substrate, whereas natural habitats include 2,780 km² of hard substrate (Rezak et al. 1990, Gardner and Beaudoin 2005, Decomworld 2013). Studies concur that the artificial habitats in the NGOM are red snapper dominated, while the natural habitats support a more species diverse system (Kasprzak and Perret 1996, Gledhill 2001, Gallaway et al. 2009).

While we do know there are differences in red snapper morphology and demographics in the NGOM east and west of the Mississippi River, we do not know why (Woods 2003, Jackson et al. 2007, Kulaw 2012, Saari et al. (in press)). Previous studies indicate that growth rates, feeding habits, nutritional condition, etc., differ substantially for red snapper inhabiting natural and artificial habitats (Saari 2011, Kulaw 2012, Schwartzkopf 2014). The goal of my research was to elaborate on recent finding by determining whether observed differences in demography are reflected in reproductive

potential of female red snapper at natural and artificial habitats on the Louisiana continental shelf.

METHODS

Sites

Red snappers from multiple sites at two distinct habitat types, natural and artificial, were examined and compared to determine reproductive relationships. All five sites are located in the NGOM 130-200 km southwest of Port Fourchon, Louisiana. The natural habitat sites included Jakkula Reef, McGrail Reef, and Bright Reef, which are situated on the edge of the Louisiana/Texas continental shelf (Figure 3.2). The artificial habitat sites, East Cameron and Eugene Island, are located on the continental shelf and both consist of toppled and standing platforms (Figure 3.2).

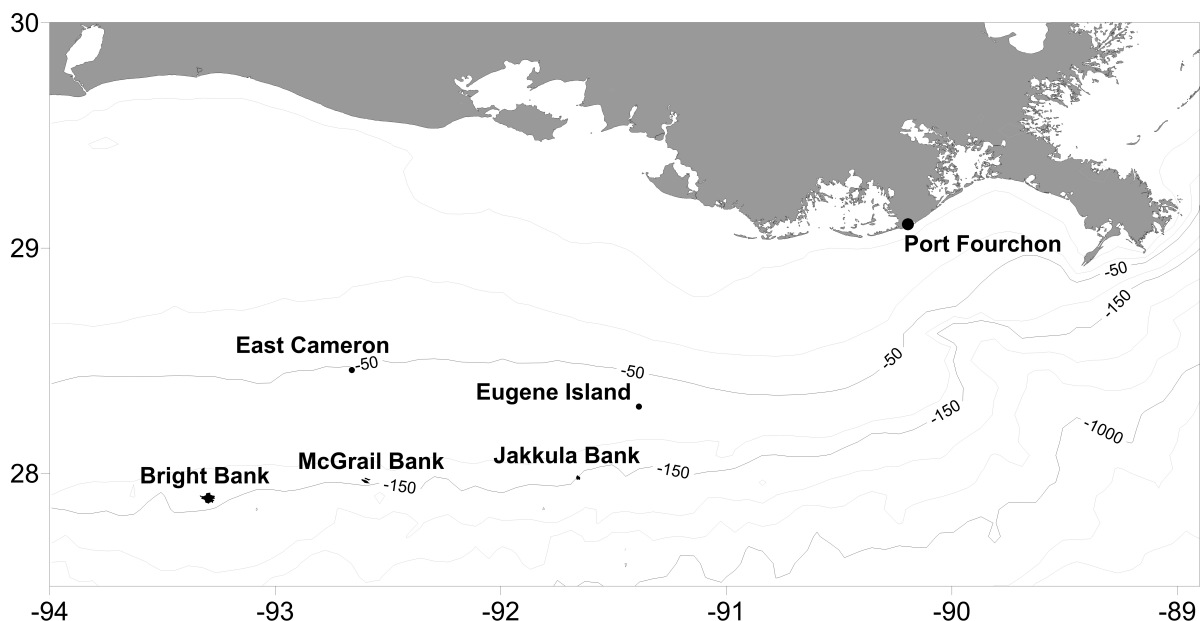


Figure 3.2 Collection sites for red snapper (*Lutjanus campechanus*). Jakkula Reef, McGrail Reef, and Bright Reef are naturally occurring shelf edge reefs; East Cameron and Eugene Island are in artificial reef planning areas that include both standing and topples platforms. East Cameron is situated on a large patch of lithified delta mud (Cowan et al. 2007).

Jakkula Reef, the most easterly site, was sampled in an earlier study and was included here to provide a benchmark for comparison between years and sites (Saari 2011, Kulaw 2012). The substrate on Jakkula Reef is composed mostly of sand and some mud with coral whips and rocky outcrops. Jakkula Reef is exposed to greater sedimentation, relative to other natural sites in this study, because of its proximity to the mouth of the Mississippi River. Also, Jakkula Reef has the highest relief at 92m. The crest of the reef is the deepest of those in the study and it has the least amount of exposed hard substrate (Table 3.1) (Gardner and Beaudoin 2005).

McGrail Reef is the smallest in area of the natural sites and has the least relief at 65m (Table 3.1). Two steep pinnacles separated by a deep valley distinguish McGrail Reef's profile. This site has both the most stony coral of the sites we sampled and supports a diverse community of reef and pelagic fishes (Gardner and Beaudoin 2005). The substrate in this area is composed of many large, rocky outcroppings. Near the pinnacles, the sea floor is covered in loose carbonate, pebble-sized debris composed of coral rubble and shells.

Bright Reef is the most westerly site (Figure 3.2); the substrate is composed mostly of coral and algae rubble. Bright Reef is the largest and shallowest site with 75m of relief (Table 3.1). Many large rocky outcroppings are found on Bright Reef, creating a prime environment for red snapper. Hard corals are found on Bright Reef, but not in the abundance seen at McGrail Reef (Rezak et al. 1990).

Table 3.1 Physical characters of sites sampled. East Cameron and Eugene Island are artificial habitats, whereas Jakkula, McGrail, and Bright are natural habitats. Values for East Cameron and Eugene Island are calculated from acoustic data. Values for Jakkula, McGrail, and Bright are taken from Gardner and Beaudoin (2005), who define relief as the height from the top of the structure to the adjacent seafloor.

Site	Seafloor depth/shallowest depth (m)	Relief (m)	Dimensions (m ²)
Jakkula Reef	160/64	92	2,890
McGrail Reef	110/45	65	13,200
Bright Reef	130/33	75	29,760
East Cameron	55/29	26	2,580
Eugene Island	75/30	45	3,137

The East Cameron site is situated within the East Cameron Oil and Gas Lease Area and is a part of Louisiana Artificial Reef Program (LARP) (Figure 3.2) (Kasprzak and Perret 1996). Since the start of the LARP, decommissioned oil and gas platforms have been moved to East Cameron and deployed as artificial habitat. The artificial habitat at East Cameron consists of both toppled and standing platforms. It has been shown previously that there are no significant differences in red snapper reproductive potential at the standing and toppled platforms (Kulaw 2012). East Cameron's artificial habitat is over twenty years old and are considered mature (Kasprzak and Perret 1996). A previous study described a two-kilometer ridge rising approximately two meters above the adjacent substrate; this is thought to be a patch of lithified delta mud (Cowan et al. 2007).

The Eugene Island site is also situated within a LARP area in the Eugene Island Oil and Gas Lease Area (Figure 3.2). The first addition of a decommissioned platform to Eugene Island was in 1992 (Kasprzak and Perret 1996). Eugene Island is also composed of toppled and standing platforms but, as at East Cameron, they are considered equivalent habitats with regards to red snapper reproduction. This site was chosen for this study for

its proximity to Jakkula Reef as well as its use in a previous red snapper study (Saari 2011, Kulaw 2012). Eugene Island was included in the analysis for this chapter as a replicate for the artificial habitat.

Sample Collection

Thirty hook or ten hook vertical longlines with alternating large and small hooks were used to harvest red snappers for research. Hooks were spaced roughly one foot apart with enough weight (approximately 4-5 kg) at the terminus to keep the hooks vertical in the water column. Four longlines were deployed for one to three hours per site per trip, depending on weather and sea condition. Single hook rods and reels were used either in conjunction with or instead of the longlines when appropriate. These methods caught a variety of species, although only red snapper were considered in this study.

All fish caught were fitted with a numbered tag and held on ice until dissection. All red snapper were measured for total length (TL, mm), fork length (FL, mm), standard length (SL, mm), total weight (TW, kg), and eviscerated body weight (EW, kg). Muscle tissue, fin clips, livers, stomachs, otoliths, and ovaries were removed at sea, frozen, and transported to either the Fisheries Science Laboratory or the Fish Age and Growth Laboratory at Louisiana State University (LSU).

Tissue Preparation

In the laboratory ovaries were thawed, blotted dry with paper towels, and weighed to the nearest 0.01 gram. The ovaries then were preserved in 10% formalin for no less than two weeks (longer if ovaries were very large). Large ovaries were punctured with a

scalpel to allow for formalin to perfuse into and fix the inner ovary before bacteria could affect the oocytes (Nieland and Wilson 1995). Red snapper ovarian lobes are developmentally symmetrical; one sample from each ovary was sufficient for histological slide preparation and characterization of the reproductive state (Collins et al. 1996). Histological slides were prepared from an ovarian cross section approximately 2 mm thick selected and prepared as follows. The ovary was visually divided into six sections; one section was chosen for sample extraction at random with a six-sided die (Appendix, A1). Cross sections were then placed in numbered histological cassettes. Fifty-six cross-sections were stored in 10% formalin solution and transported to the LSU School of Veterinary Medicine for slide preparation. The remainder of the histological slides were processed and made in the LSU Fish Age, Growth, and Histology Laboratory.

Slide Processing

Ovarian tissues were processed using a Leica ASP6025 tissue processor and then embedded in paraffin wax at a Leica EG 1150H embedding station. Tissues were then cut to 4µm with a Leica RM2125 RTS microtome. Cut tissues were attached to clean microscope slides in a warm water bath and transferred to a hot plate to dry. The slides then were stained and counterstained with hematoxylin and eosin, respectively, in a Leica ST5020 Multistainer. Lastly, a Leica CV5030 Coverslipper applied cover slips with Permount.

Oocyte Stage Analysis

All ovarian slides from peak spawning season (June, July, and August) were examined for maturity with an Olympus BX41 microscope at 40x magnification.

Oocytes were classified according to the four stages of oocyte development given by Wallace and Selman (1981) and Brown-Peterson et. al. (2011): primary growth, cortical alveoli, vitellogenesis, and hydrated (Table 3.2, Appendix, A2-A4).

Based upon the designations developed by Wallace and Selman (1981) red snapper are heterochronal batch spawners with different stages of oocytes simultaneously occurring within the ovary during the spawning season . The presence of vitellogenic oocytes during spawning season was the benchmark for maturity in female red snappers (Nieland and Wilson 1993, Woods 2003, Jackson et al. 2005, Brown-Peterson et al. 2011).

Table 3.2 Biological explanation and histological characteristics of the progression of oocyte maturation for female heterochronal and asynchronous batch spawning marine teleosts. (Adapted from: Wallace and Selman 1981, Woods 2003, McMillan 2007, Brown-Peterson et al. 2011, and Kulaw 2012)

Phase	Biology	Histological Characters
Primary Growth (PG)	Centrally located large basophilic nucleolus; surrounded by squamous scale-like follicle cells; presence of only PG oocytes during the spawning season is indicative of immaturity or regenerative stage (Appendix, A2)	Oocytes small 20-200 μm ; little space between oocytes; oocytes dark purple in color with lighter core
Cortical Alveoli (CA)	The release of gonadotropin initiates the formation of “yolk vesicles”; small lipid droplets form around the nucleolus; CA oocytes are present immediately before vitellogenesis, but do not explicitly imply spawning capability (Appendix, A3)	Oocytes medium 200-500 μm ; purple in color, but lighter than PG; light core; lipids dissolve during preservation, leaving light ring of vesicles around nucleus

(Table 3.2 continued)

Phase	Biology	Histological Characters
Vitellogenic (V)	Vitellogenin is released by the liver and transported to the ovaries via the bloodstream where it is cleaved into yolk proteins; the yolk proteins enter the oocytes by passing between the follicle cells, creating “lipid yolk droplets”; nucleus moves toward the animal pole; late V or early hydration appears homogeneous due to yolk coalescence; presence of V oocytes clearly reveals maturity (Appendix, A3)	Oocytes medium to large 0.50-1 mm; light purple/pink; distinctly lighter than CA or PG oocytes; grainy in appearance; appears homogeneous just before hydration
Hydrated (H)	Rapid increase in size due to water uptake- in morning for red snapper- immediately before spawning; homogeneous cytoplasm; formation of yolk plate; nucleus becomes difficult to identify; H oocytes indicate imminent spawning (Appendix, A4)	Largest oocyte size 1-2 mm; light pink; ‘squished balloon’ appearance due to dehydration during fixation; no nucleus visible
Postovulatory Follicles (POF)	Follicle walls collapse into empty space previously occupied by H oocyte creating network of distinct folds; red snapper POF degenerate within 24 hours; clearly folded POF indicate a female that has very recently spawned (Appendix, A5)	Small; light red/pink; consists of many collapsed folds
Atresia (A)	Oocyte is reabsorbed by phagocytosis; follicle layer becomes fragmented and the oocyte disintegrates; can be present in any stage but commonly occurs at the end of spawning season; also can be linked to nutritional condition (Appendix, A6)	Any size; discolored; dissolution of cell wall; oocyte still recognizable but clear break down at edges

Maturity was not assessed outside the spawning season because only primary growth and cortical alveoli stages are present, regardless of maturity. Maturity was also established by the presence of hydrated oocytes, postovulatory follicles (POF), or atretic oocytes within spawning season (Appendix, A4-A6). Ovaries with hydrated oocytes underwent further analysis to determine batch fecundity estimates, spawning frequency, and annual fecundity.

POF in ovaries indicate recent spawning within the previous 24 hours (Table 3.2) (Hunter and Macewicz 1985a). A POF is a layer of epithelial granulosa cells plus an outer layer of connective thecal tissue left from a hydrated oocyte after ovulation and spawning (Woods 2003, McMillan 2007). A POF is identifiable as a structure of collapsed folds that degrades quickly (within 24 hours).

Oocytes that are mature but not spawned usually are reabsorbed through a process called atresia. Atresia may be present at all stages of spawning, though it occurs more commonly at the cessation of spawning season (Hunter and Macewicz 1985b, Brown-Peterson et al. 2011). Because atresia is not present in immature fish, it can be an indicator of maturity, as well as the end of an individual's spawning season.

Gonadosomatic Index

Gonadosomatic indices (GSI) were calculated for all female red snapper sampled. GSI is a ratio of ovary mass to eviscerated body mass:

$$GSI = \frac{Ovary\ Mass\ (g)}{Eviscerated\ Body\ Mass\ (g)} \times 100$$

Increases in GSI indicate spawning readiness and potential investment of energy in reproduction (Davies 1956, Collins et al. 1996). GSI values greater than one denote that

females are capable of spawning and peak spawning season in red snapper (Collins et al. 1996).

Size and Age at Maturity

Reproductively active female red snapper were sorted into both 50 mm TL size classes and age groups. The individuals in class were then analyzed for 50% and 100% maturity.

Batch Fecundity Estimate

Those red snapper ovaries with hydrated oocytes, based upon histological evidence, were further examined to estimate batch fecundity estimates (BF). BF is estimated gravimetrically from counts of the number of hydrated oocytes in a known weights of a subsample of ovarian tissue (Hunter et al. 1985). That number is then extrapolated to the weight of the entire ovary to obtain the approximate number of eggs that female would have spawned during the next spawning event, as follows:

$$BF = \left(\frac{\# \text{ hydrated oocytes}}{\text{sample wt (g)}} \right) * \text{whole ovary wt (g)}$$

Three 0.03-0.05 g subsamples were taken randomly from among the six regions of the ovaries and placed on a microscope slide in a 3:7 glycerin-water solution. Hydrated oocytes, distinguished by their greater size and translucence, were then visually counted under a compound microscope (Olympus BX41, 10x magnification) (Render and Wilson 1992, Wilson and Nieland 1994, Collins et al. 1996). The three BF estimates were averaged for each individual female.

Spawning Frequency

Red snapper are indeterminate batch spawners, periodically releasing batches of eggs over the course of a spawning season. In reproductively mature female ovaries, immature oocytes are continually developing into batches of mature oocytes. Each histological slide analyzed is only a snapshot of that female's reproductive season; this makes efforts more difficult when trying to estimate annual fecundity. This is why spawning frequency (SF) is used to estimate mature oocyte production.

SF estimates the average number of days between consecutive spawning events or the number of days for all females in the population to spawn one time (Hunter and Macewicz 1985a, Nieland et al. 2002). There are three different methods for determining SF: the POF method, the hydrated oocytes method, and the time-calibrated method (Hunter and Macewicz 1985a, Fitzhugh et al. 1993, Wilson and Nieland 1994, Nieland et al. 2002, Woods 2003, Kulaw 2012). Each method has its own assumptions, but firstly they assume that red snapper are indeterminate batch spawners. Methods for determining SF are as follows:

- 1) The POF method uses the total number of females with evidence of POF (day 1). After a fish spawns POF can only be recognized clearly for 24 hours (Table 3.2) (Wilson and Nieland 1994, Nieland et al. 2002). The spawning fraction is the proportion of females currently spawning on any given day. The inverse of the proportion of females with POF, or spawning fraction, represents the average number of days between spawning events for the population during the spawning season. This method is difficult to use with a small data set; however, it is preferable to the hydrated method in most cases.

$$Spawning\ Fraction_{POF\ method} = total\ \# \ day\ 1 / total\ \# \ mature$$

$$\begin{aligned} SF_{POF\ method} &= 1 / Spawning\ Fraction_{POF\ method} \\ &= total\ \# \ mature / total\ \# \ day\ 1 \end{aligned}$$

2) The hydrated oocyte method uses the ratio of the number of females with hydrated oocytes (day 0) to the total number of mature females in the sample population (Table 3.2) (Hunter and Macewicz 1985a). Spawning fraction is calculated in the same way as the POF method. The inverse of the proportion of females with hydrated oocytes, or spawning fraction, represents the average number of days between spawning events for the population during spawning season. The hydrated oocyte method assumes that all hydrated oocytes will be released as eggs within the next few hours. This method is not preferred because of potential sampling bias; females with hydrated oocytes can only be found during a short period of the day.

$$Spawning\ Fraction_{H\ method} = total\ \# \ day\ 0 / total\ \# \ mature$$

$$\begin{aligned} SF_{H\ method} &= 1 / Spawning\ Fraction_{H\ method} \\ &= total\ \# \ mature / total\ \# \ day\ 0 \end{aligned}$$

3) The time-calibrated or TC method takes into account the numbers of females with either hydrated oocytes or POF. The TC method assumes ovaries that show oocytes with either yolk coalescence (late vitellogenesis) or hydration (day 0) will progress to spawning within the next 10-12 hours (Nieland et al. 2002). This

method must also take into account the assumption of both the hydrated and POF methods. Adding the day 0 and day 1 females and then dividing by the total number of mature females, calculates spawning fractions. The inverse of the proportion of spawning fraction represents the number of days between spawning events for the population during spawning season. I used the TC method because the greatest amount of data is used to determine SF, especially when sample sizes are small.

$$Spawning\ Fraction_{TC\ method} = ((total\ \# \ day\ 0 + total\ \# \ day\ 1)/2) / total\ \# \ mature$$

$$SF_{TC\ method} = 1 / Spawning\ Fraction_{TC\ method}$$

$$= total\ \# \ mature / ((total\ \# \ day\ 0 + total\ \# \ day\ 1)/2)$$

To estimate the spawning events per season, I used a 150-day spawning season (Woods 2003).

$$Spawning\ events\ per\ season = \frac{150\ days}{SF}$$

Annual Fecundity

Annual fecundity (AF) is the estimate of the number of eggs a female red snapper release during an entire spawning season. AF is calculated from the SF, a 150-day spawning season, and BF (Nieland and Wilson 1993, Woods 2003).

$$AF = \left(150\ days / SF \right) \times BF$$

AF was calculated for each female for which a BF was estimated.

Statistical Analysis

All statistical tests were done with the Statistical Analysis System (SAS v 9.3, Cary NC); statistical significance was determined at $\alpha=0.05$ for all tests. Analysis of variance (ANOVA) was used to compare sex ratios of red snappers between habitats. Multivariate analysis of variance (MANOVA) was used to compare red snapper habitat-wide demographic characteristics: mean age, percent maturity, GSI, mean TL, mean TW, and seasonal bottom water temperature. Linear regression was applied to calculate red snapper weight-length relationships and analysis of covariance (ANCOVA) was used to compare those relationships between habitats.

ANOVA was also used to test mean GSI values by month, habitat, and month*habitat; year, water temperature, and site(habitat) ran as random components. The slice function was used to compare month*habitat for like months.

A final ANOVA, with only females sampled during peak spawning months, tested mean GSI values by habitat, size class, and habitat*size class; year, water temperature, and site(habitat) ran as random components. The slice function was used to compare month*size class for the same size classes.

Random Forest

Random forest analyses were applied to estimate the relative importance of independent variables on the prediction of a dependent variable. 1000 regression trees were grown based upon subsets of the data and at each node a subset of the independent variables were used. The random forest algorithm then calculates how much error decreases when a variable in the regression trees is used as a predictor while other

variables in the tree are held constant. The predictive decreases in error are then averaged and plotted by the Gini index (Liaw and Wiener 2014). The first random forest run used habitat as the dependent variable and used GSI, TL, age, maturity, and month as the predictors of to which habitat an individual belongs. The second random forest run used maturity as the dependent variable and used GSI, TL, site, age, habitat, and month as the predictors of whether an individual was mature or immature.

RESULTS

A total of 718 red snapper were collected at natural (Jakkula Reef, McGrail Reef, and Bright Reef) and artificial (East Cameron and Eugene Island) habitats from 2011 to 2013; 346 (48%) red snapper were collected during peak spawning months (June, July, and August). Of the specimens collected during spawning season, 161 (46%) were females. The sex ratio of females during peak spawning season was 50:50 at the artificial habitat, but less than 50:50 at the natural habitat (Table 3.3).

Table 3.3 Sex ratios of red snapper (*Lutjanus campechanus*) sampled during peak spawning months (June July, August). There is no statistically significant difference of sex ratios between the habitats (ANOVA, $P < 0.1420$).

Habitat	Female	Male	% Female
Artificial	98	99	50%
Natural	63	86	42%

Red snapper ages ranged from 2 to 17 years (M. Kormanec, personal communication). The youngest red snapper was found at the artificial habitats; the oldest red snapper was found at the natural habitats (Table 3.4). The most frequent age for red snapper at artificial habitats was 5 years old, whereas the most frequent age for red snapper at natural habitats was 6 years old (Figure 3.3). There was a lack of 2 and 3 year

olds at the natural habitat and a lack of red snapper over 9 years old at the artificial habitats. Red snapper mean age was significantly different between the habitats (MANOVA, $p < 0.0001$) (Table 3.4).

Table 3.4 Descriptive demographic characteristics of female red snapper (*Lutjanus campechanus*). Least squares means \pm standard error or N (%).

* $p < .05$ MANOVA comparing characteristics between the habitats.

Characteristic	Artificial Habitat	Natural Habitat
N (females) †	98	63
Age Range (years) †	2-9	4-17
Mean Age (years)	5 ± 1	$7 \pm 1^*$
Total Length (mm)	529 ± 11	$580 \pm 14^*$
Total Weight (kg)	2.18 ± 0.14	$2.71 \pm 0.17^*$
% Mature (females only)	50 (52%)	62 (98%) *
Gonadosomatic Index	0.63 ± 0.09	$1.87 \pm 0.12^*$
Bottom Water Temperature (C°)	24 ± 1	$23 \pm 1^*$

† Not included in the MANOVA model-used only to show trends.

Length-weight analysis of TL and EW was done on all female red snapper caught during peak spawning months by habitat type. A predictive regression, $TL = 1.15(SL) + 28.09$, fitting TL to standard lengths (SL, mm) was used to estimate missing TL ($r^2 = 0.8816$, slope = 1.15, intercept = 28.09). EW and TL from all sites were \log_{10} transformed and EW was regressed on TL. Both regressions were significant ($P < 0.0001$) and both regression coefficients showed excellent fit ($r^2 < 0.90$) (Figure 3.4).

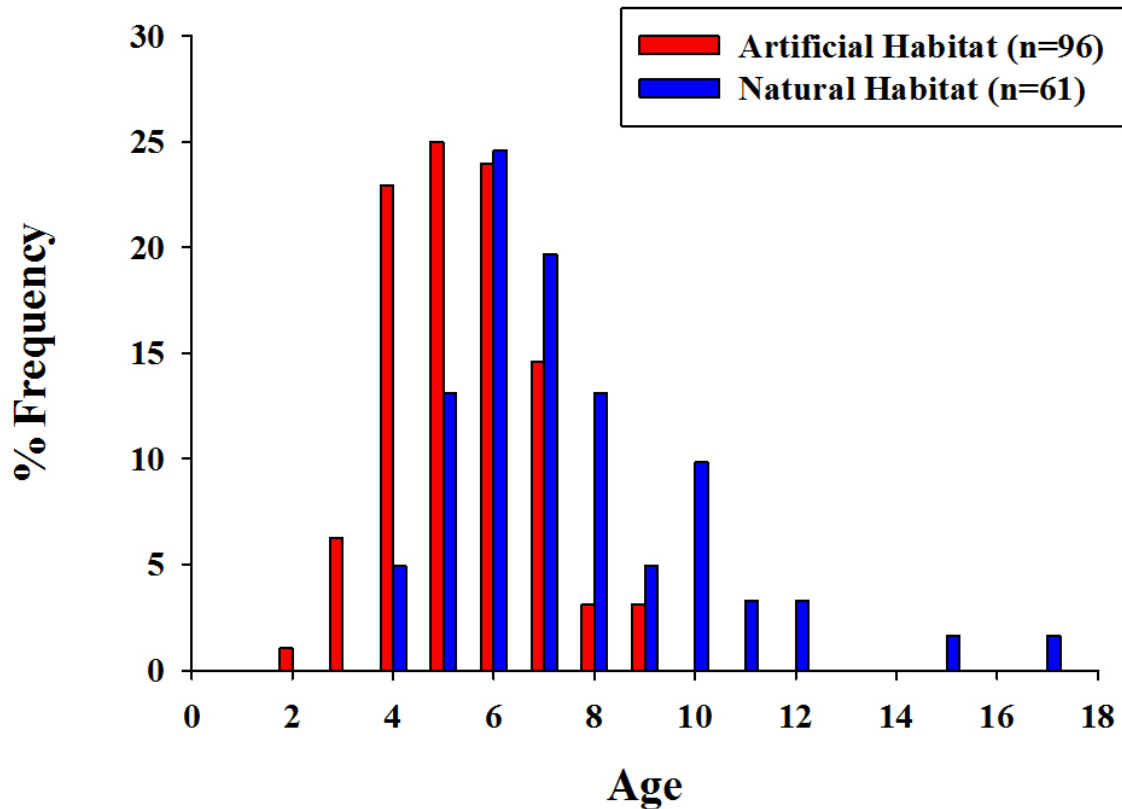


Figure 3.3 Age frequencies of female red snapper (*Lutjanus campechanus*) caught during peak spawning months (June, July, and August). Four individuals could not be aged due to missing or damaged otoliths.

An analysis of covariance (ANCOVA) showed that the length-weight relationships of females caught during spawning season at both the habitats have slopes different from zero ($p < 0.0001$) and the intercepts are significantly different from each other ($p < 0.0001$). However, the slopes are not significantly different between habitats ($p = 0.6459$) (Figure 3.4). The results of the ANCOVA were the same when the interaction term was removed.

Among female red snapper sampled during spawning season, TL ranged from 301 to 793 mm; the largest individual was found on natural habitat while the smallest was found on artificial habitat (Table 3.4). When TL was split into 50 mm groups, frequency of female red snapper lengths on natural habitat peaked at 550 mm and at 500 mm on

artificial habitat (Figure 3.5). The artificial habitat in general had higher frequencies of smaller red snapper than the natural habitats (Figure 3.5). The red snapper sampled during spawning season at natural habitats had a significantly different mean TL than the red snapper at artificial habitats (MANOVA, $p = 0.0015$) (Table 3.4)

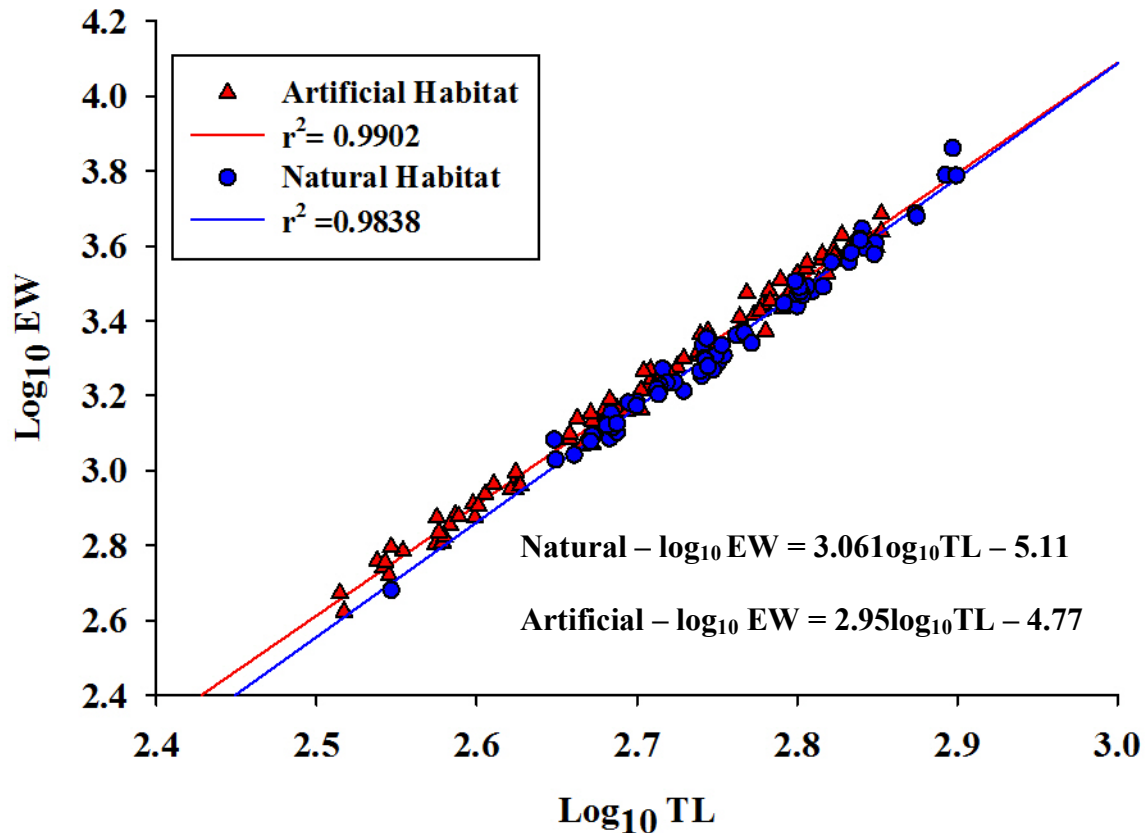


Figure 3.4 Total length-eviscerated weight regressions for female red snapper (*Lutjanus campechanus*) sampled at each habitat during spawning season (June, July, August). EW = eviscerated body weight (g); TL = total length (mm). (Artificial habitat: slope = 2.95, intercept = -4.77) (Natural habitat: slope = 3.06, intercept = -5.11)

TW of red snapper sampled during spawning season ranged from 0.45 kg to 7.65 kg. The heaviest red snapper was found on natural habitat while the leanest was found on artificial habitat. Mean TW of red snapper sampled during spawning season was significantly different between natural and artificial habitats (MANOVA, $p = 0.0172$) (Table 3.4).

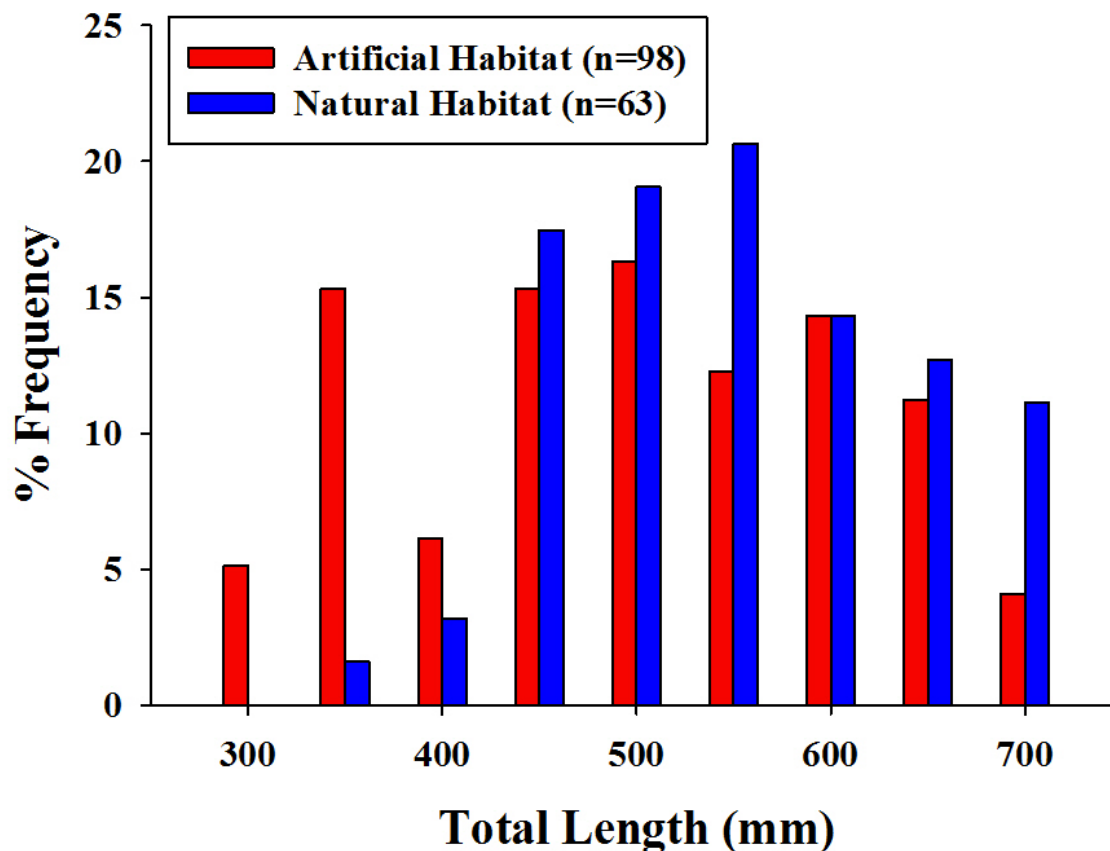


Figure 3.5 Length frequencies of female red snapper (*Lutjanus campechanus*) caught during peak spawning months (June, July, and August).

Gonadosomatic Index

Red snapper GSI ranged from 0.14 to 7.59; the lowest GSI was found in red snapper collected on artificial habitat, and the highest at natural habitat. Mean GSI during peak spawning months (June, July, and August) was significantly higher at the natural habitats than on artificial habitats (MANOVA, $p < 0.0001$) (Table 3.4).

There is a clear increase in mean GSI at both habitats during May and mean GSI peaked at both habitats in June, although the peak at natural habitats is much greater than at artificial habitats. Mean GSI declined earlier at the artificial habitats than at the natural habitats (Figure 3.6).

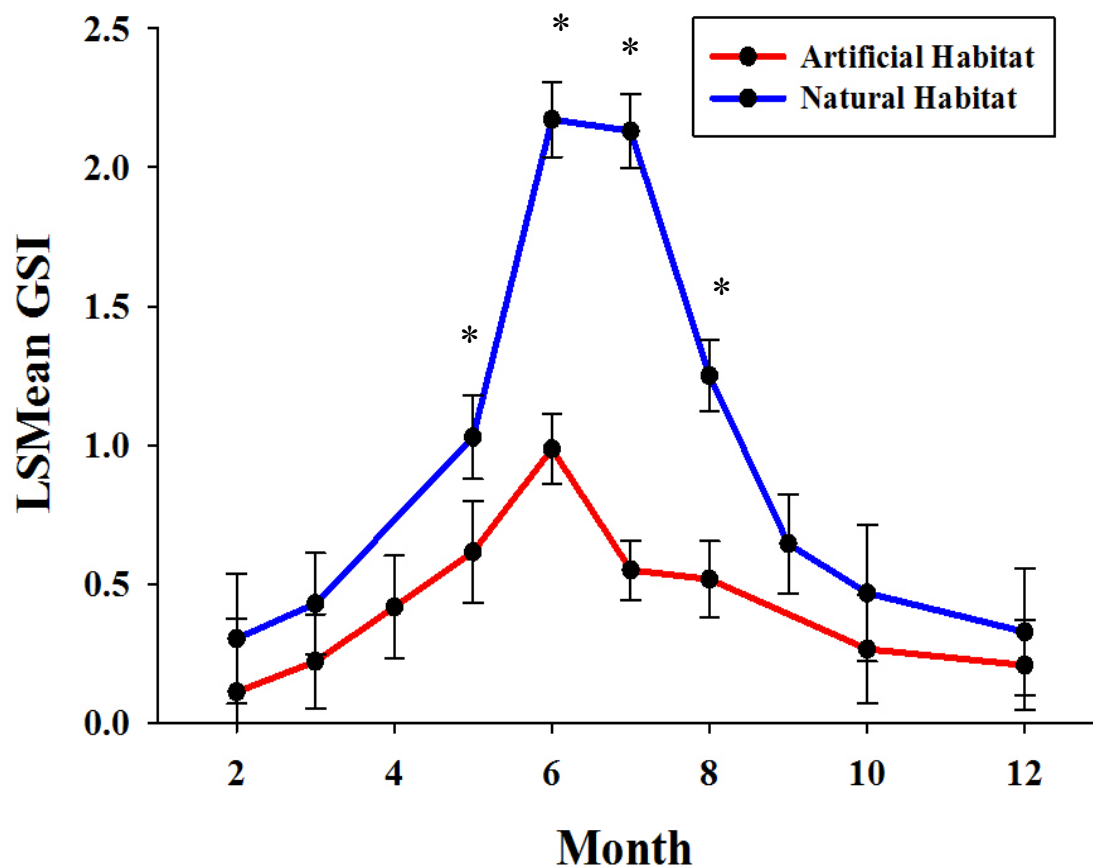
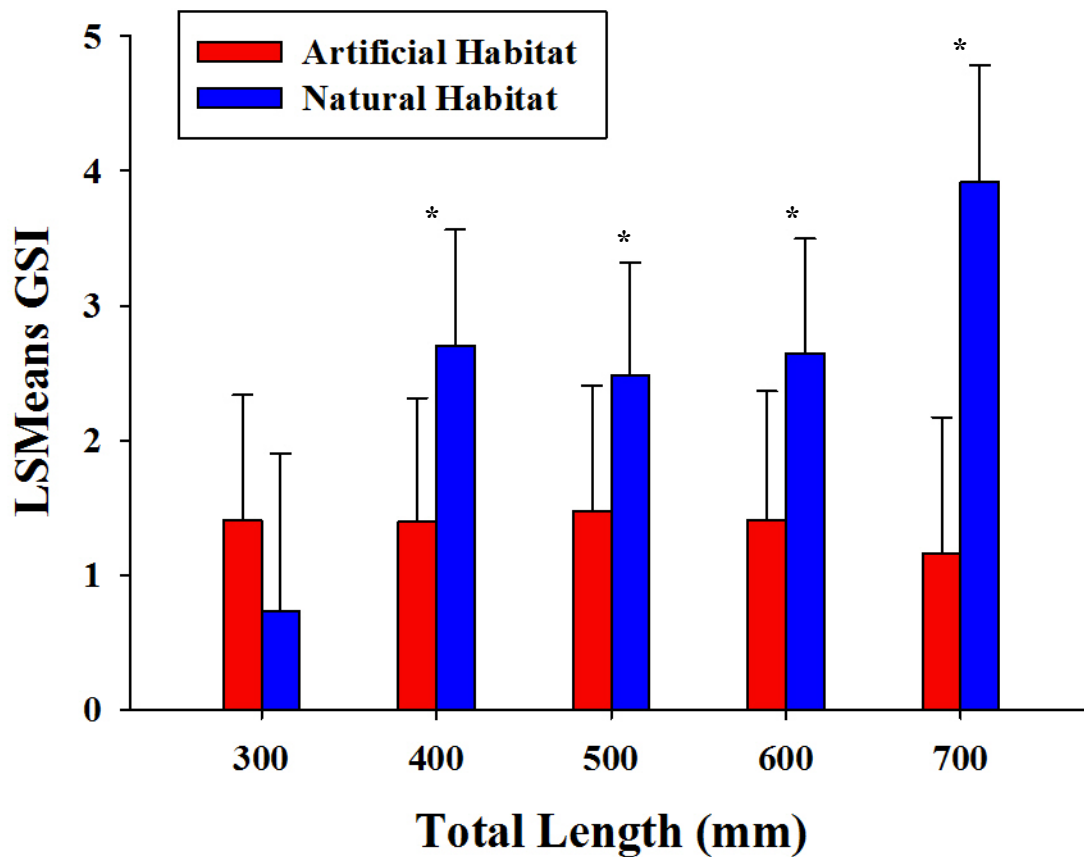


Figure 3.6 Mean monthly gonadosomatic indices (GSI) for female red snapper (*Lutjanus campechanus*) at both habitats; verticle bars represent standard errors of monthly means. Least squared means are used instead of actual means to give a better representation of compared values. * indicates a significant difference in mean GSI between habitats at that month (ANOVA, $p < .05$).

Monthly mean GSI were compared between habitats using an ANOVA. The splice function was used to identify interactions between the habitats by month. Mean GSI for female red snapper was only significantly different between habitats during May ($p=0.0194$), June ($p<0.0001$), July ($p=0.0008$), and August ($p=0.0025$) (Figure 3.6). The patterns at natural habitats correspond with the traditional red snapper spawning season, whereas the artificial habitats are truncated.

To address the possibility that natural habitat had larger and more fecund red snapper than the artificial habitat, mean GSI was assessed by habitat and 100 mm size class (SC). An ANOVA was used to compare mean red snapper mean GSI at SC between habitats during peak spawning season (June, July, and August). The splice function was used to make direct comparisons between habitats by SC. The mean red snapper GSI for the smallest SC was not significantly different between habitats ($p=0.4224$). However, mean GSI was significantly different between all other SCs (400 mm $p=0.0090$, 500 mm $p=0.0464$, 600 mm $p=0.00054$, 700 mm $p=0.0124$) (Figure 3.7).



3.7 Mean gonadosomatic indices (GSI) by total length (TL) class for female red snapper (*Lutjanus campechanus*) at artificial and natural habitats; verticle bars represent standard errors of means. Only females caught during spawning season are represented. Least squared means (LSMeans) are used instead of actual means to give a better representation of compared values. * indicates a significant difference in mean GSI between habitats at that total length (ANOVA, $p<.05$).

When female red snapper are further split into months of collection and SC, the trend shows that females at natural habitats have higher mean GSI by SC, for the majority of the spawning season (Figure 3.8). The differences in mean female GSI between the habitats are generally stronger for the larger SC, whereas the smaller SC are more similar in mean GSI. Figure 3.8 also shows a lack of the smallest SC at the natural habitats during July and August, and a lack of the largest SC at the artificial habitat during August.

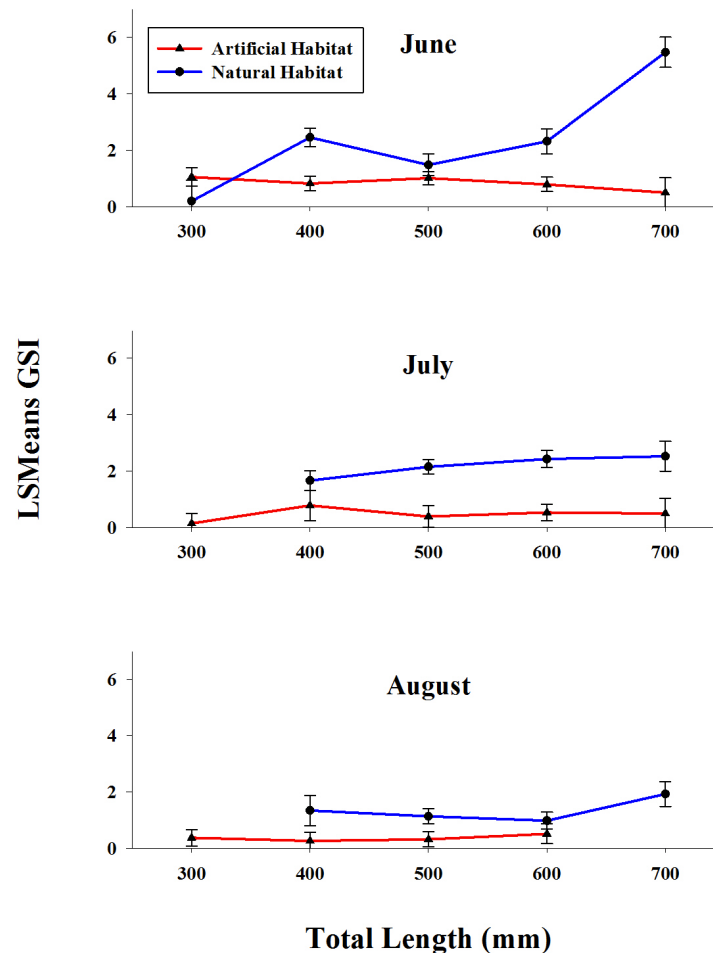


Figure 3.8 Mean gonadosomatic indices (GSI) by total length for female red snapper (*Lutjanus campechanus*) at both habitats; verticle bars represent standard errors of means. June, July, and August were used to represent the peak of spawning season. Least squared means (LSMeans) are used instead of actual means to give a better representation of compared values.

Maturity

A total of 161 female red snapper were assessed for sexual maturity. Female red snapper not caught during peak spawning months were not assessed for maturity because mature regenerating females can be mistaken for immature females. The smallest specimen, 301 mm TL, to reach maturity was from artificial habitat and was 3 years old. The smallest mature female red snapper from natural habitat was 446 mm TL and the youngest was 4 years old (Table 3.5 and 3.6).

Every female red snapper caught at natural habitat was mature with the exception of one 352 mm, 6 year old individual, which is small-at-age in general for a female red

Table 3.5 Maturity ratios by size class of female red snapper (*Lutjanus campechanus*) sampled during spawning months (June, July, August). One maturity value is missing for the artificial habitat because the ovary was lost before histological analysis was complete. TL= Total length (mm).

Size Class	Artificial Habitat		Natural Habitat	
TL (mm)	% Mature	N	% Mature	N
301-400	38	19	0	1
401-500	43	21	100	13
501-600	50	26	100	23
601-700	68	25	100	17
701+	50	6	100	9

Table 3.6 Percent maturity by age of female red snapper (*Lutjanus campechanus*) sampled during spawning months (June, July, August). One maturity value is missing for the artificial habitat because the ovary was lost before histological analysis was complete.

Age	Artificial Habitat		Natural Habitat	
Years	% Mature	N	% Mature	N
2	0	1	-	-
3	17	6	-	-
4	55	22	100	3
5	39	23	100	8
6	48	23	93	15
7	64	14	100	12
8	100	3	100	8
9+	100	3	100	15

snapper (Figure 3.9 and 3.10). The red snapper in natural habitats reached 100% maturity at 401-500 mm SC and at 4 years of age (Table 3.5 and 3.6). Red snapper at the artificial habitat reached 50% maturity at the 501-600 mm SC and 7 years of age (Table 3.5 and 3.6, Figure 3.9 and 3.10). 100% maturity at artificial habitats was not reached in any SC, but was reached at 8 years of age (Table 3.5 and 3.6). Total percent maturity of female red snapper was significantly different between the habitats (MANOVA $p < 0.0001$, Table 3.4).

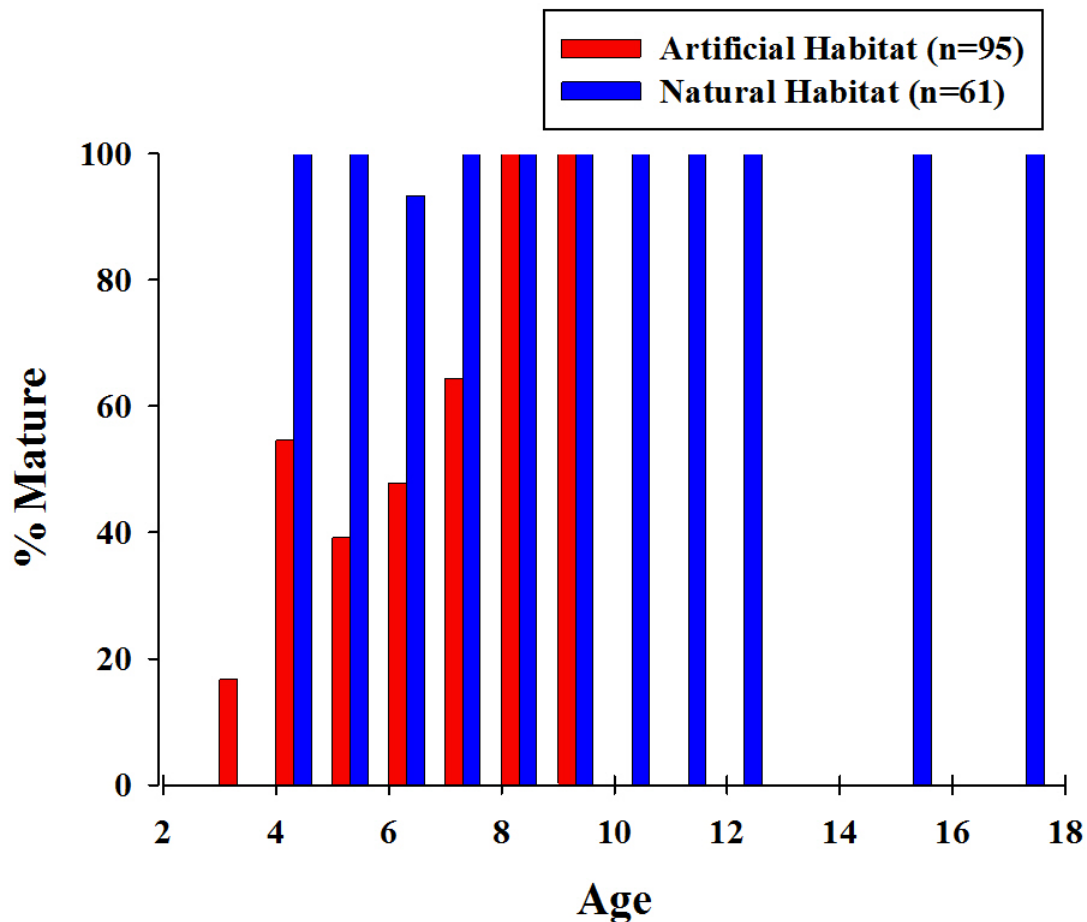


Figure 3.9 Percent of mature female red snapper (*Lutjanus campechanus*) caught during peak spawning months (June, July, and August) by age. Determined by oocyte stage analysis of histological slides. Four individuals could not be aged due to missing or damaged otoliths. Maturity of one fish could not be determined because of a misplaced ovary.

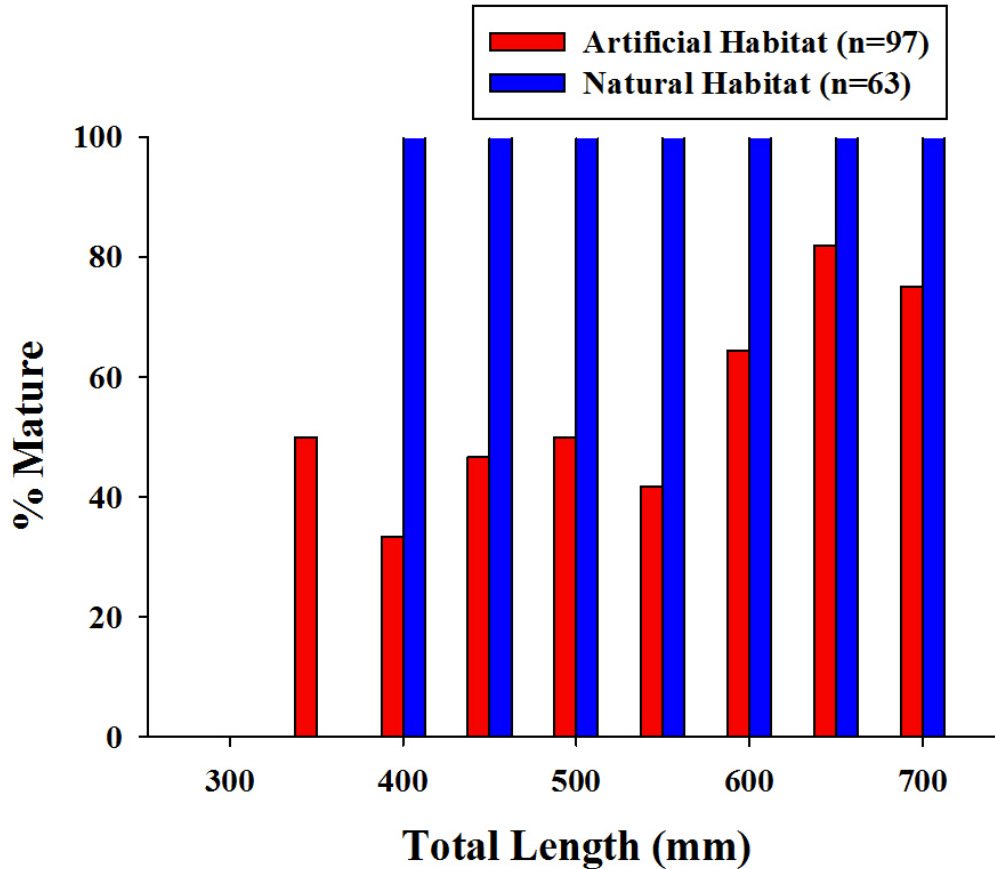


Figure 3.10 Percent of mature female red snapper (*Lutjanus campechanus*) caught during peak spawning months (June, July, and August) by 50 mm size class. Determined by oocyte stage analysis of histological slides. Maturity of one fish could not be determined because of a misplaced ovary.

Spawning Indicators

Only nine red snapper ovaries had hydrated oocytes, seven from artificial habitat and two from natural habitat. The smallest individual with hydrated oocytes was from artificial habitat and was 4 years old and 351 mm TL. The largest individual with hydrated oocytes came from natural habitat, was 10 years old, and 789 mm TL. Red snappers from natural habitat had a higher percentage of ovaries with POF, despite having a smaller sample size (Table 3.7).

Table 3.7 Descriptive fecundity variables of female red snapper (*Lutjanus campechanus*) sampled during spawning season (June, July, August). Means \pm SD or N (%).

Characteristic	Artificial Habitat	Natural Habitat
Ovaries with Hydrated Oocytes	7 (5%)	2 (3%)
Postovulatory Follicles in ovaries	5 (4%)	14 (22%)
Batch Fecundity Estimate (eggs/batch)	41,878 \pm 48,027	704,563 \pm 693,573
Annual Fecundity Estimates (eggs/season)	1,369,334 \pm 1,600,920	26,323,179 \pm 26,147,495

Batch Fecundity

Batch fecundity (BF) was estimated for female red snappers with hydrated oocytes; BF ranged from 6,991 to 1,194,993 eggs per batch. The mean BF was higher at natural habitats than at artificial habitats (Table 3.7). Batch fecundity estimates for the red snapper on artificial habitats were generally low regardless of either size or age. The BF estimates for red snapper on artificial habitats are all at the low end of BF for red snapper (Appendix, Table A1); otherwise there are no discernable patterns when BF is plotted by age and TL (Figure 3.11).

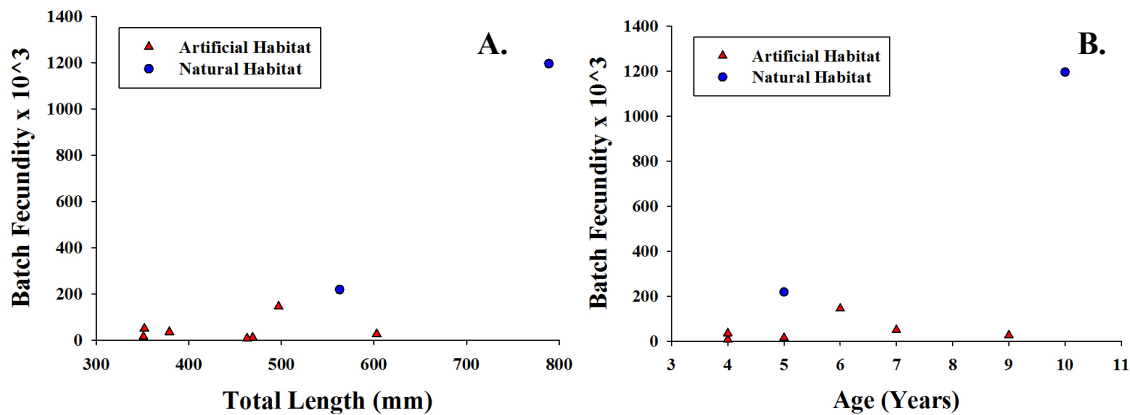


Figure 3.11 Batch fecundity of female red snapper (*Lutjanus campechanus*). A = total length (mm), B = Age (years).

Spawning Frequency

Red snapper spawning frequency (SF) was estimated with three different methods (Table 3.8) (Fitzhugh et al. 1993, Nieland et al. 2002). Given a 150-day spawning season and with the time calibrated method to estimate SF, 39 spawning events would have been expected at natural habitat, whereas only 33 spawning events were estimated at artificial habitats (Table 3.8). Mature female red snapper at natural habitats are predicted to be 4% more likely to be spawning during any give day in peak spawning season with the TC method (Table 3.8).

According to the POF method, red snappers at artificial habitats spawned 15 times per season while red snapper at natural habitats spawned 34 times per season (Table 3.8). A greater proportion of mature female red snapper at natural habitats are likely to be spawning during any day in spawning season than at artificial habitats according to the POF method (Table 3.8).

The hydrated method predicts female red snapper at artificial habitats have 7 more spawning events per season than red snapper from natural habitats (Table 3.8). The hydrated oocyte method also found that a higher proportion of mature female red snapper would be spawning on any day during the spawning season at artificial habitats than at natural habitats (Table 3.8). Spawning frequency was not compared at age and TL between habitats because of insufficient data.

Annual Fecundity

Annual fecundity for red snapper was calculated with the TC method for spawning frequency and with batch fecundity estimates. Red snapper at artificial habitats

Table 3.8 Spawning frequency calculation methods of female red snapper (*Lutjanus campechanus*) sampled during spawning season (June, July, August). Spawning events per season were calculated with a 150 day spawning season defined by Woods 2003

	Artificial Habitat	Natural Habitat
Mature Females	50	62
Postovulatory Follicles (day 1)	5	14
Ovaries with Hydrated Oocytes (day 0)	7	2
Late Vitellogenic Oocytes (day 0)	10	16
<u>POF Method</u>		
Spawning Fraction (% of mature females spawning during any day in spawning season)	10%	23%
Spawning Frequency Estimate (days between spawning)	10	4.4
Spawning Events per Season	15	34
<u>Hydrated Oocyte Method</u>		
Spawning Fraction (% of mature females spawning during any day in spawning season)	34%	29%
Spawning Frequency Estimate (days between spawning)	2.9	3.4
Spawning Events per Season	51	44
<u>Time Calibrated Method</u>		
Spawning Fraction (% of mature females spawning during any day in spawning season)	22%	26%
Spawning Frequency Estimate (days between spawning)	4.5	3.9
Spawning Events per Season	33	39

had lower mean annual fecundity than at natural habitats (Table 3.7). The large standard deviation is most likely due to a small sample size.

Random Forest Analysis

A random forest analysis was used to measure the relative importance of GSI, TL, age, maturity, and month on the prediction of individual red snapper inhabiting natural or artificial habitats. Site was left out of this analysis because each site was already assigned a habitat and this would bias the model toward site being the most effective predictor. The random forest found that GSI was the most important factor followed by TL and age to predict which habitat an individual would be found (Figure 3.12).

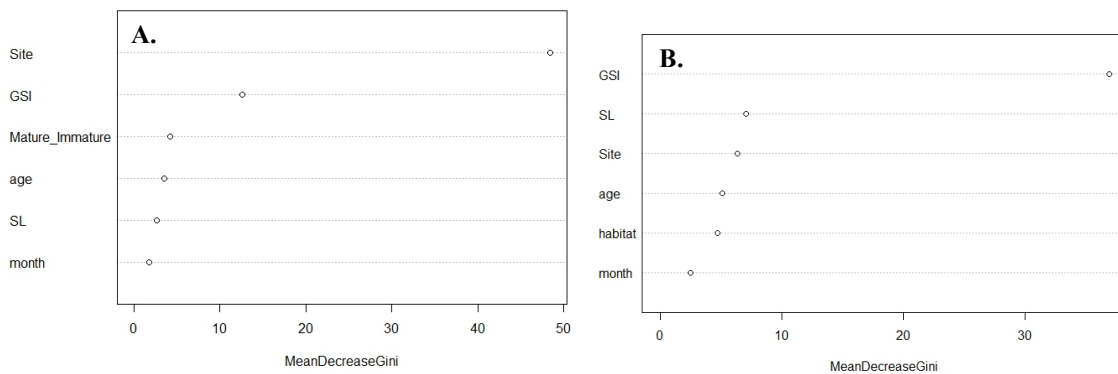


Figure 3.12 Gini index results of a random forest analysis of predicting red snapper habitat and maturity. A. Random forest trained to focus on predicting habitat type. B. Random forest trained on maturity

A second random forest was used to investigate the relative importance of GSI, TL, site, age, habitat, and month on the prediction of whether a female red snapper was mature or immature. Site was left in this analysis because using both site and habitat removes the previously stated conflict. This random forest also found GSI to be the most

important predictor for maturity followed by TL and site, although TL, sit, age, habitat, and month were all relatively close in importance.

DISCUSSION

The population of red snapper in this study may not represent the red snapper population in the entire Gulf of Mexico. This data set only contains individuals of ages 2-17 (2-9 years at artificial habitats and 4-17 years at natural habitats), which is truncated on the upper end and underrepresented at the lower end. There is also a lack of representation in the smaller size classes at natural habitats and a complete absence of any red snapper less than 300 mm total length (TL). However, this data set does represent the populations of red snapper at the habitats where they were caught. Red snapper experience several ontogenetic shifts in their life and their presence on the habitats at age was consistent with previous studies (Patterson et al. 2001, Workman et al. 2002, Gallaway et al. 2009). Therefore, the reproductive results presented in this study do accurately reflect the populations of female red snappers at these habitats.

I found that female red snapper at natural habitats have greater reproductive potential than female red snapper at artificial habitats. Based upon the results of the random forest analysis I concluded that GSI was the most important variable in predicating habitat and maturity. Throughout this study GSI has shown differing trends between the two habitats and has proven to be significantly higher at natural habitats during all spawning months. Before this study little work had been done on natural habitats in the NGOM. We now know that the reproductive potential of red snapper appears to differ significantly with habitat. A young, small female red snapper at a

natural habitat is more likely to be mature during peak spawning season than a red snapper at artificial habitat regardless of size or age.

Female red snappers at natural habitats are lighter-at-size than female red snappers at artificial habitats. My interpretation of the ANCOVA results show that the intercepts for the weight-length relationships were significantly different between the two habitats, whereas the weight-length slopes were not significantly different. These results contradict a previous study that found red snapper at natural habitat were heavier, but shorter, than red snapper at artificial habitat (Saari 2011, Kulaw 2012).

The weight-length relationship for red snapper can represent condition or nutritional well being by showing patterns of how the weight and length relationship changes throughout the population (Pitcher and Hart 1983). Females exhibit a lowered weight-length condition during spawning season due to use of the energy resources used in reproduction (Pitcher and Hart 1983). According to the TC method for calculating SF, female red snapper at natural habitats are spawning more frequently than red snapper in artificial habitats. The increased spawning frequency at natural habitats may explain why the weight-length relationship indicates lower condition at natural habitats during spawning season.

During the spawning season red snapper must allocate energy for either somatic growth or reproduction. Reproductive effort and somatic growth both use a large amount of energy in teleost species (Schreck et al. 2001). The process by which a fish “chooses” how to allocate energy is still unknown. Yet, better nutritional condition is linked to increased reproductive output, whereas diminished nutritional condition and stress are known to decrease reproductive output (Schreck et al. 2001, Marteinsdottir and Begg

2002, Morgan 2004). The red snapper at the natural habitats are in better nutritional condition than the red snapper at artificial habitats (Schwartzkopf 2014). Larger energy stores could represent a surplus of energy, allowing the red snapper at natural habitats to allocate more energy to reproduction.

The red snapper at artificial habitats also exhibited a truncated spawning season compared to the red snapper at natural habitats. In most teleost species, GSI fluctuates throughout the year and peaks during the spawning season (Davies 1956, Brown-Peterson et al. 2011). In red snapper a mean GSI of >1 has been associated with spawning readiness (Grimes 1987, Collins et al. 1996). During this study, the mean GSI for red snapper at artificial habitats was above 1 for June only, lending evidence to a truncated spawning season at artificial habitats.

With a truncated spawning season and lower mean GSI during peak spawning months, the female red snapper at artificial habitats likely do not have the same potential for egg production as the red snapper at natural habitats. Thresher (1983) found a negative correlation between reproductive success and intraspecies competition. Interpretations of data from concurrent community studies have shown that populations at artificial habitats are dominated by red snapper and fish populations at natural habitats are far more diverse (Langland, personal correspondence). Intraspecies competition could be one of the factors driving the lower reproductive potential evident at the artificial habitats.

The female red snapper at natural habitats matured both sooner and at smaller sizes than the female red snapper at artificial habitats. Several studies agree that 100% maturity for red snapper is reached between 6 and 8 years of age, supporting the maturity

rates seen at the artificial habitats of this study (Woods 2003, Woods et al. 2003, Kulaw 2012). However, red snapper at natural habitats reached 100% maturity at age 4, earlier than previously seen in the NGOM (Appendix, Table A1). Previous maturity-at-length studies agree with my results of red snapper at artificial habitats, but the red snapper at natural habitats are 100% mature at smaller sizes than previously reported (Collins et al. 1996, Woods 2003, Fitzhugh et al. 2004, Jackson et al. 2007, Kulaw 2012) (Appendix, Table A1). Brulè et al. (2010) found red snapper on the Campeche Banks in Mexico to be 50% mature at 314 mm TL and 100% mature at 526 mm TL. The maturity patterns for red snapper at natural habitats in the NGOM more closely resemble red snapper collected on the Campeche Banks than previous studies in the NGOM (Brulé et al. 2010). Early maturation is normally a sign of juvenescence. Yet, since there is the evidence that the populations of red snapper at natural habitats are in better nutritional condition juvenescence is not likely to be the reason for early maturation that I found on the artificial habitats. These results should be interpreted with caution, however, because of the low sample size of young and small female red snapper. Nevertheless, the sample as a whole is likely representative of the populations at these particular locations because younger red snapper have not yet recruited to these habitats. The small sample size does not negate the fact that female red snapper at natural habitats were mature when female red snapper of the same age and size at artificial habitats were not.

It is possible that the female red snapper at artificial habitats are experiencing skip spawning. A female teleost that has reproduced in previous seasons but “chooses” not spawn in the current season is skip spawning (Morgan and Lilly 2006, Rideout et al. 2006). Skip spawning is not based on size or age, but is associated with poor condition,

poor diet, and intraspecies competition (Thresher 1983, Trippel 1995, Morgan and Lilly 2006, Rideout et al. 2006). A fish that is in poor nutritional condition will use its energy stores for somatic growth instead of reproduction, because survival is deemed more important than reproduction (Rideout et al. 2006). Red snapper at natural habitats are in better nutritional condition, have a richer diet, and are experiencing less intraspecies competition than red snapper at artificial habitats (Langland, personal correspondence, Schwartzkopf 2014). At natural habitats female red snapper are maturing earlier because they can “afford” to trade somatic growth for reproductive potential. Conversely, the red snapper at artificial habitats are in poor nutritional condition forcing a tradeoff between reproduction and somatic growth.

Liver condition is linked to reproductive maturity and is based on liver size relative to body size (Morgan 2004). The livers of female fish produce the hormone vitellogenin (Morgan 2004, McMillan 2007); which is what triggers the maturation of cortical alveoli oocytes into vitellogenic oocytes, signaling maturity during spawning season (McMillan 2007). Liver indices normally are highly correlated with GSI (Morgan 2004). Studies have shown that female fish with good liver condition are more likely to be mature than fish with poor liver condition, regardless of size or age (Silverstein et al. 1997, Marteinsdottir and Begg 2002, Morgan 2004). Female red snapper at natural habitats could be exhibiting accelerated maturation because they are in better nutritional condition and have higher liver conditions as well (Schwartzkopf 2014).

Batch fecundity (BF) estimates are normally a powerful gauge of reproductive potential. Unfortunately, there were very few female red snapper collected with hydrated oocytes in their ovaries. The BF estimates for red snapper at artificial habitats were

lower than previously recorded BF estimates (Collins et al. 1996, Woods 2003, Kulaw 2012) (Appendix, Table A1). However, the BF estimates for red snapper at natural habitats correlate well with previously reported ranges of BF (Collins et al. 1996, Woods 2003, Kulaw 2012) (Appendix, Table A1). Reduced fecundity has been linked to poor diet and nutritional condition in teleost species (Marteinsdottir and Begg 2002, Rideout et al. 2006). The low BF estimates at the artificial habitats may be attributed to the poorer nutritional condition of red snapper in these habitats (Schwartzkopf 2014).

Spawning frequency of red snapper between habitats varied a great deal when using the POF and hydrated oocyte methods. The best estimates of SF are found when a population can be sampled over a 24-hour period during spawning season because we had limited sampling time, this could have biased the results. Two of the previous studies of red snapper reproduction use the TC method to estimate spawning frequency; this method will be used to compare the results of this study.

Spawning frequency estimates for red snapper at artificial habitats have been reported as 4.2 to 7.0 days between spawning events (Woods 2003, Kulaw 2012) (Appendix, Table A1). The spawning frequency estimate for red snapper at natural habitats was 8.5 days between spawning events (Kulaw 2012), whereas spawning frequency at natural habitats in this study was 3.9 days between spawning events. Spawning events per season at natural habitats are also much higher in this study than in previous studies (Kulaw 2012)(Appendix, Table A1). At artificial habitats the previous ranges of spawning events per season compare well with the value from this study (Woods 2003, Kulaw 2012) (Appendix, Table A1). The conflicting results are likely due to the lack of representation of small and young red snapper females and the lack of

information from natural habitats.

Annual fecundity (AF) estimates suggest that female red snapper at natural habitats are contributing more eggs per female to the NGOM population proportionally than the red snapper at artificial habitats. The AF at natural habitats in this study was 26.3 ± 26.1 million eggs per year. The variation for this AF estimate is so large because it is based on BF estimates from two red snapper (1.2 and 44.8 million eggs per year), thus this AF estimate likely may not reflect the true AF at natural habitats. Kulaw (2012) found red snapper AF at natural habitats to be 5.8 million eggs per year, but this estimate is based on only one observation (Appendix, Table A1). It is clear more work needs to be done at natural habitats in the NGOM to better understand red snapper AF.

AF estimates for red snapper from artificial/natural habitat in Florida ranged from 0.01 to 59.7 million eggs per season (Collins et al. 1996). The red snapper AF estimates from an earlier study is 23.3 million eggs per season in Louisiana and 13.4 million eggs per season in Alabama (Woods 2003). Kulaw (2012) estimated AF of red snapper at toppled platforms to be 3.4 ± 2.5 million eggs per season and at standing platforms to be 3.4 ± 2.2 million eggs per season. The red snapper AF estimate at artificial habitats was 1.4 ± 1.6 million eggs per year, lower than in previous studies (Appendix, Table A1). Obviously AF of red snapper is highly variable in this study as well in other studies. This study shows red snapper at natural habitats exhibit higher AF estimates than the red snapper at artificial habitats. These results agree somewhat with previous studies.

This study generally reflects that the red snapper at natural habitats are reproducing when they are smaller, younger, and over their lifetime will be more fecund than red snapper at artificial habitats. Pitcher and Hart (1983) said “the ages at first

maturity is then a balance between the advantages of being big and the increasing chance of dying”. At natural habitats, red snapper have higher GSI, batch fecundities, spawning frequencies, and annual fecundities than red snapper at artificial habitats. As it stands the red snapper at natural habitats seem to use less energy on the advantages of being big and instead focus on the advantages of being reproductively prolific.

These findings are fundamentally different from what we previously knew about red snapper at natural habitats. Although a large number of individual red snapper inhabit artificial habitats in the NGOM, the apparently are not capable of devoting as much of their stored energy towards reproduction as the red snapper occupying natural habitats (Schwartzkopf 2014). I believe that red snapper on artificial habitats in the NGOM are less important to the productivity of the stock in the NGOM than conspecific found on the natural shelf edge reefs.

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CHAPTER 4: GENERAL CONCLUSIONS

The majority of all research on red snapper, including reproduction, has been based upon red snapper collected on artificial habitats; however, there is a population of red snapper on natural shelf edge reefs that exhibit large differences in demographics from those on artificial habitats. My study shows that female red snappers on natural habitats have higher reproductive potential than female red snappers at artificial habitats in the NGOM. Previous studies have shown that populations of red snapper are demographically distinct within the NGOM (Jackson et al. 2007, Kulaw 2012, Sluis et al. 2012, Saari et al. (in press)). My research has taken demographic surveys a step further and analyzed red snapper reproduction at specific habitats within the red snapper population of the northwestern Gulf of Mexico. The implications from this study change what we previously knew about the potential reproduction of the species.

Shipp and Bortone (2009) hypothesized that red snappers were habitat limited before the addition of oil and gas platforms into the Gulf of Mexico, and that the addition of these artificial habitats has relieved a bottleneck that prevented red snapper from rising above a certain level of stock size. My research contradicts this premise by showing that red snapper at artificial habitats are unlikely to be contributing to the reproductive potential of the stock as much as historical accounts have claimed. Until we understand what proportion of the red snapper population resides on natural habitats compared to artificial habitats, we will not fully comprehend what reproductive impact either habitat has on the population as a whole.

There are major dissimilarities between natural and artificial habitats that contribute to differences in the reproductive potential of red snappers at the two habitats. Red snapper at natural habitats are maturing at younger ages and at smaller sizes, while also having a higher GSI and less truncated size and age distribution. Age and size at maturity are indicators of population size and a truncated spawning season is indicative of lowered reproductive success (Trippel 1995). It is well known that larger, older mature female red snappers are more fecund than smaller and younger females (Collins et al. 1996, Jackson et al. 2007, Kulaw 2012). A general lack of old and large mature females tends to lower the reproductive value of populations of female red snappers at artificial habitats; even the largest or oldest females at artificial habitats I studied were are not 100% mature. In addition, the females with hydrated oocytes collected in this study that were found at natural habitats had significantly higher batch fecundities than similar females at artificial habitats, again calling into question the function of artificial reefs in the western Gulf of Mexico.

The shelf edge reefs are unique habitats that predate artificial habitats by tens of thousands of years. These natural habitats and others like them are likely the historical centers of abundance for red snapper and other tropical reef associated species (Goodyear 1995, Gledhill 2001). Adding oil and gas platforms to the NGOM apparently caused a redistribution of the red snapper population, but new evidence suggests that this change may not have had a positive influence. It is possible that with more research, countergradient growth rate variation may contribute differences in reproductive potential presented found in this study. Countergradient variation is present when individual populations of the same species exhibit differences in physical processes (growth or

reproduction) along environmental gradients, but do not exhibit phenotypic differences (Conover 1990, Conover and Present 1990). Latitudinal temperature clines are the most studied mechanism for countergradient variation, but there is evidence of other types of gradients possibly accounting for countergradient variation (Trussell 2002, Kokita 2004). It is important to note, however, that geostrophic flow on the Louisiana shelf in response to large volume of Mississippi River moving westward creates south and southwesterly flow over the shelf edge reefs that keep the reefs in the “tropics” despite their latitude (Merrell and Vázquez 1983).

The physical abiotic seasonal differences in the water column (temperature, salinity, turbidity) between the shelf edge reefs and artificial reefs could also be contributing to the tradeoffs between somatic growth and reproductive potential, although this was not addressed in the current study. Other possible reasons for the discrepancies are: fishing pressure, predation, available prey, and interspecies competition. Each of these issues present an area of possible variation in red snapper populations between habitats and should be explored and addressed more thoroughly in future studies.

My personal conclusions from this research are that the shelf edge reefs are important to the red snapper population in the NGOM. The natural habitats I studied should be protected from fishing pressure and considered for further examination to understand their role in the NGOM ecosystem. Also, red snappers at artificial habitats are neither as productive nor as healthy when compared to red snapper at natural habitats. The addition of artificial habitats to the NGOM has served to aggregate populations of red snappers, but these populations do not contribute to the future of the NGOM red snapper at the same magnitude as the red snappers at the natural habitats.

Female red snapper at artificial habitats may have lowered reproductive potential due to high intraspecies competition causing high competition for limited food resources, forcing a tradeoff between somatic growth and reproductive potential for some female red snapper. Also, unfortunately, there are likely fewer large, old females because of increased fishing pressure at artificial habitats. Without the large, old, highly fecund females, those habitats cannot contribute to the population as effectively as natural habitats. The next step for understanding the populations of red snapper in Louisiana is to determine the proportion of red snapper resident at natural and artificial habitats and apply the reproductive differences found in this study. This would allow us to understand how much each population is contributing to future populations. As for recommendations, the series of shelf edge reefs on the Louisiana/Texas continental shelf should be protected for the sake of the red snapper populations in the NGOM and the state of the red snapper population should no longer be assessed solely based upon research done at artificial habitats. If there is one thing that this research has discovered, it is that we have more to learn about red snapper reproduction in the NGOM.

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APPENDIX: SUPPERLMENTARY FIGURES AND TABLE

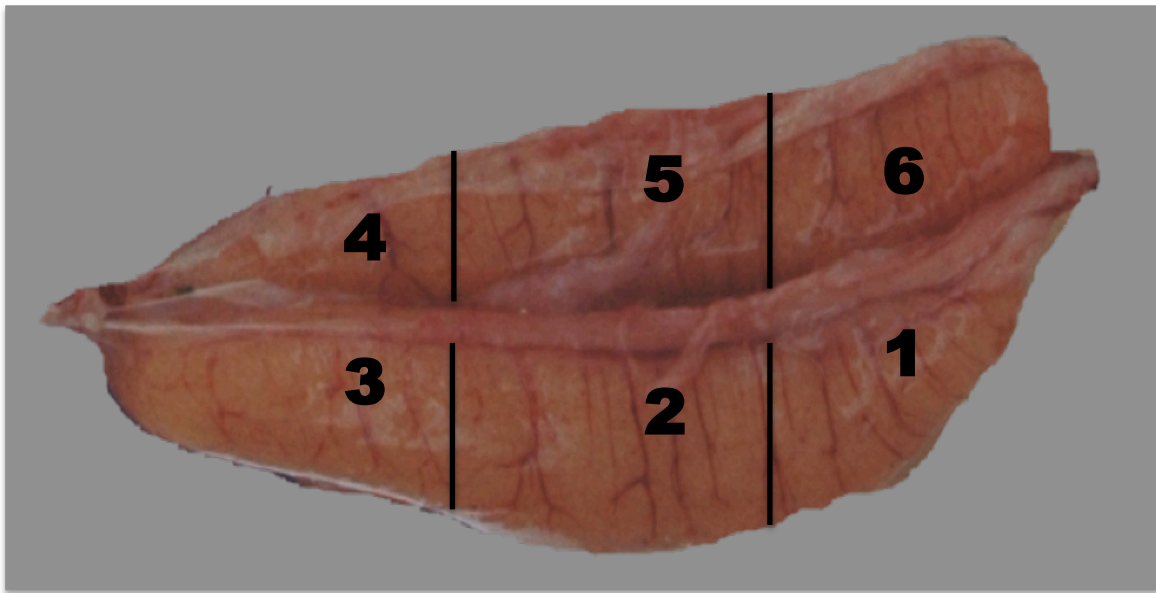


Figure A1. Image of a whole red snapper (*Lutjanus campechanus*) ovary. Numbers correspond to visually divided areas for random sampling.

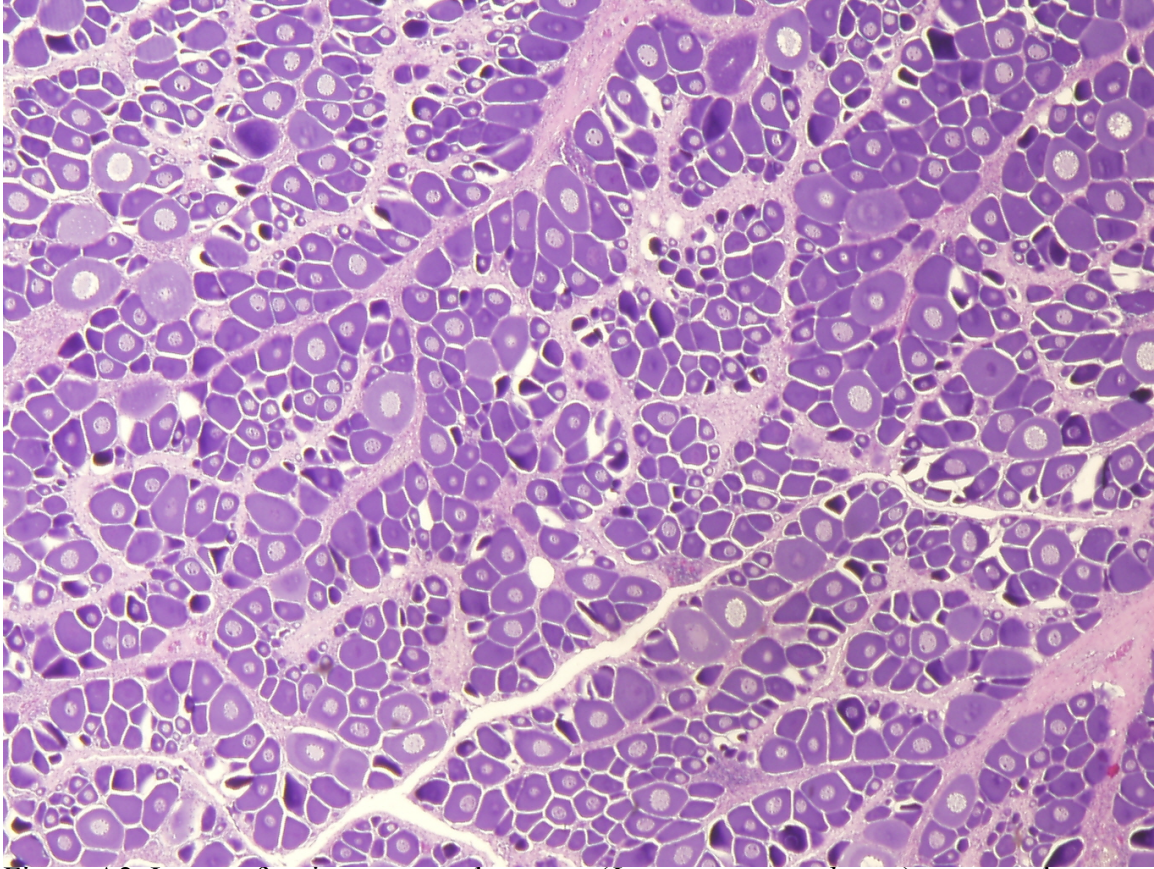


Figure A2. Image of an immature red snapper (*Lutjanus campechanus*) ovary, only primary growth oocytes are present. The lack of vitellogenic oocytes indicates immaturity.

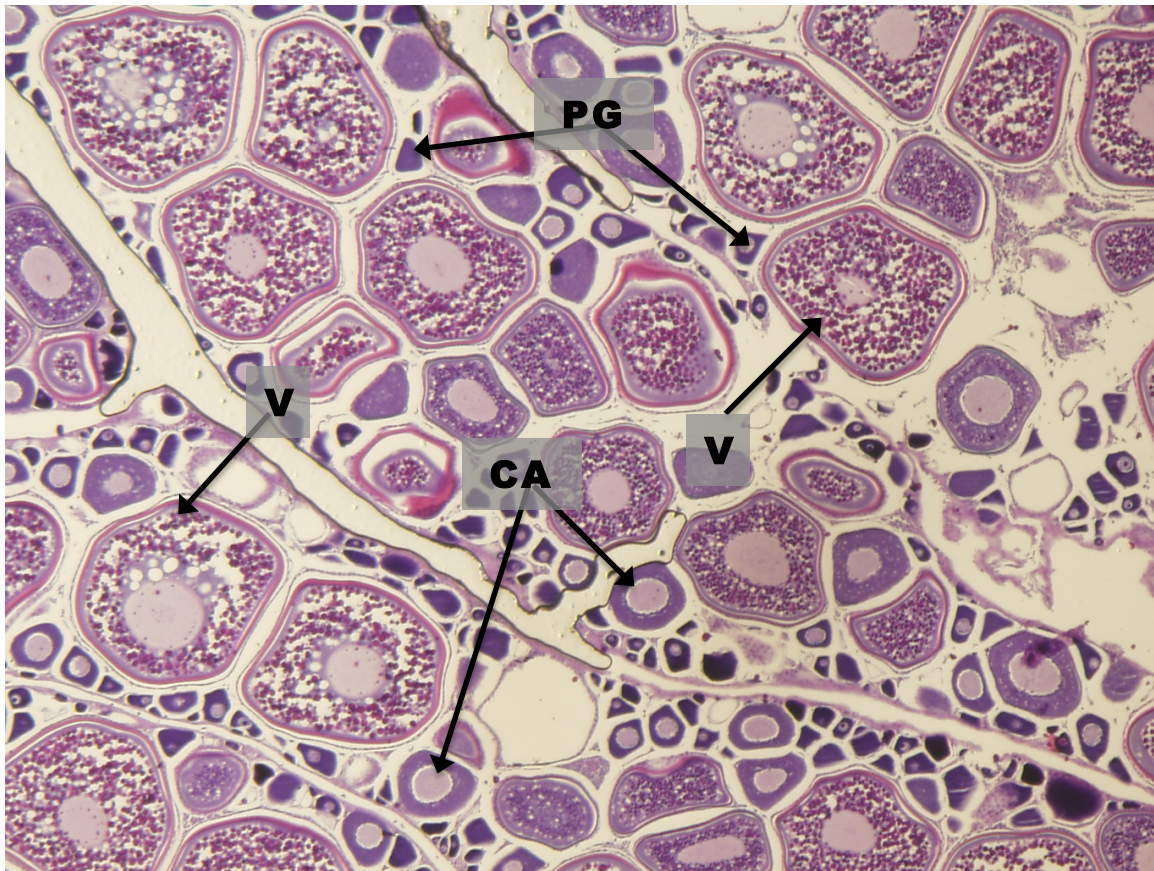


Figure A3. Image of a mature red snapper (*Lutjanus campechanus*) ovary section, three stages of oocytes are present: PG is primary growth, CA is cortical alveoli, and V is vitellogenic. The presence of vitellogenic oocytes indicates maturity.

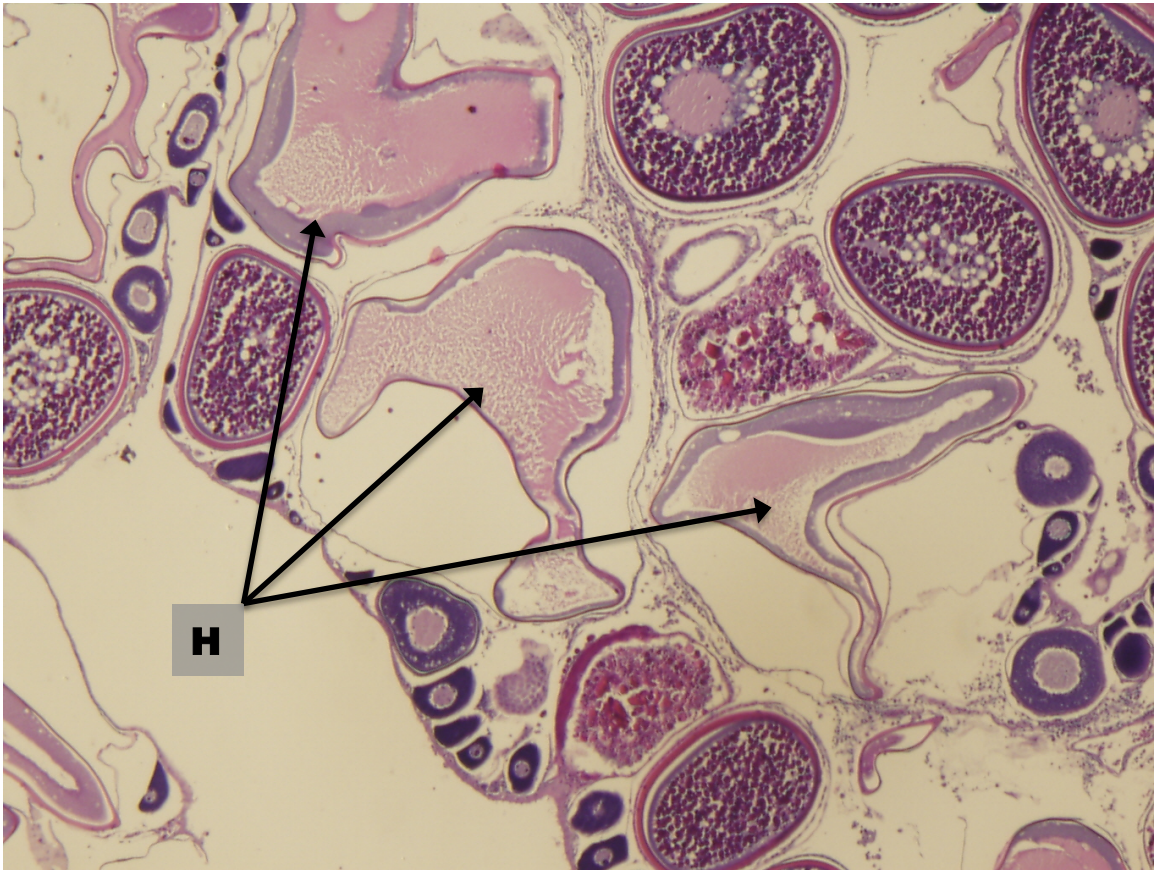


Figure A4. Image of a hydrated red snapper (*Lutjanus campechanus*) ovary section. Hydration is the final stage of oocyte maturation and red snapper hydrate oocytes immediately before releasing eggs. H is a hydrated oocyte. Irregular shape results from dehydration during fixation.

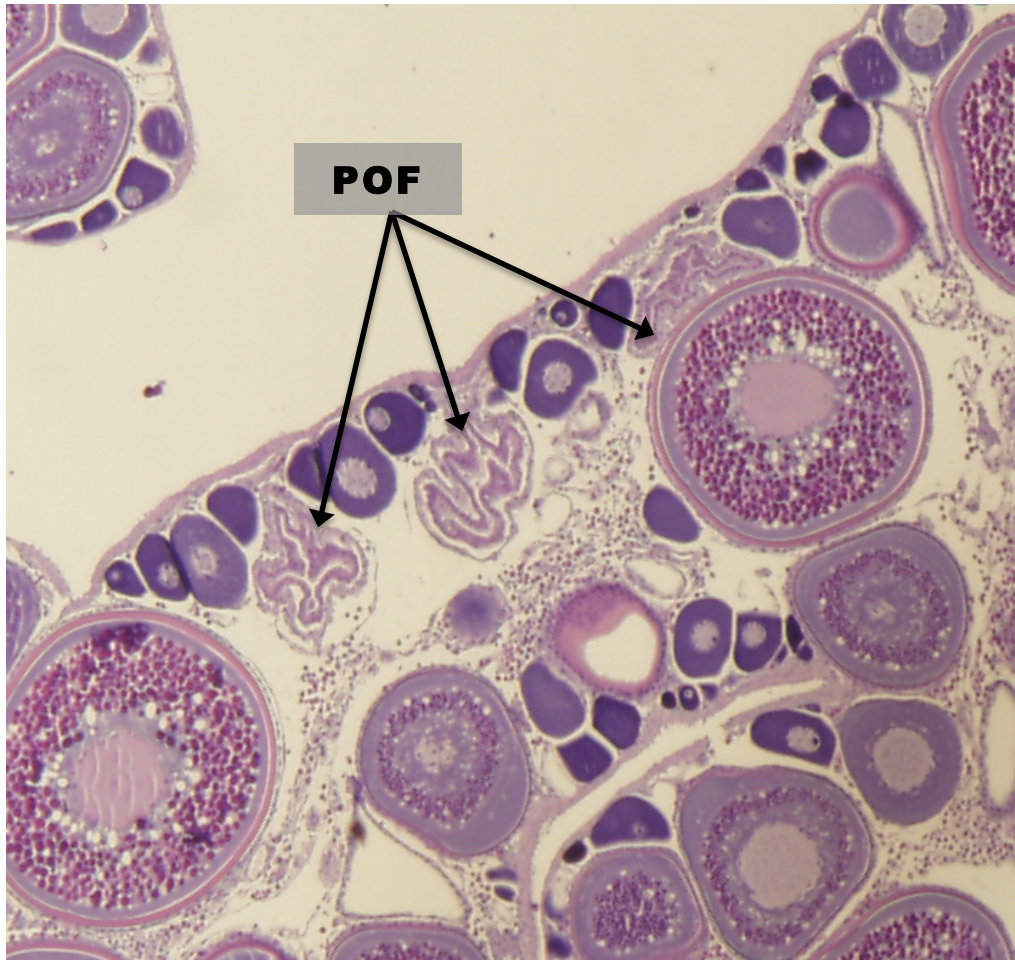


Figure A5. Image of a recently spawned red snapper (*Lutjanus campechanus*) ovary section. The presence of postovulatory follicles (POF) indicates the release of hydrated oocytes within the last 24 hours. POF are reabsorbed after approximately 24 hours.

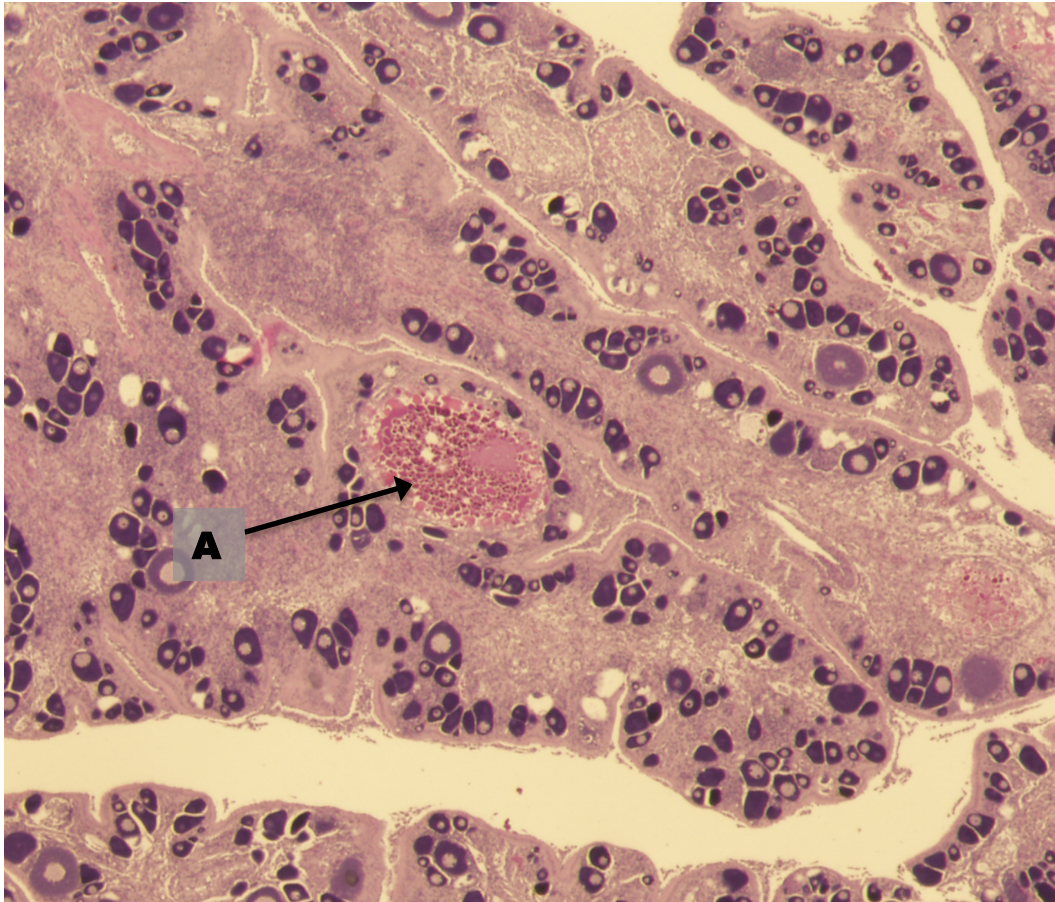


Figure A6. Image of a red snapper (*Lutjanus campechanus*) ovary section with atretic oocytes (A). Atresia can be present during the spawning season, but is most common as the end of spawning. It is characterized by the dissolution of the cell walls and cell contents.

Table A1. Results of this study and past studies of red snapper (*Lutjanus campechanus*) reproductive biology in the Gulf of Mexico. BF = batch fecundity. SF= spawning frequency. AF = annual fecundity. FL = fork length. TL= total length.

* these values denote 75% maturity.

Source	Habitat	50% Mature at age (years)	100% Mature at age (years)	50% Mature at length (mm)	100% Mature at length (mm)	BF (eggs per batch)	SF (spawning events per year)	AF (eggs per season)
Collins et al. 1996	Commercial and recreational landings Panama, FL	-	-	-	-	458 to 1,704,736	21 to 35	11,613 to 59,665,760
Woods 2003	Commercial and recreational landings; AL and LA	AL: 2 LA: 2	AL: 5 LA: 7	AL: 275 FL LA: 300 FL	AL: 450 FL LA: none	AL: 304,996 LA: 643,812	AL: 44 LA: 36	AL: 13,401,861 LA: 23,243,560
Fitzhugh et al. 2004	Commercial and recreational landings; East =FL, AL, MS West = LA, TX	-	East: 6 West: 8	East: 300 FL* West: 350 FL*	East: 600 FL West: 650 FL	-	25 to 17	-
Brulé et al. 2010	Commercial landings; NW Campeche Bank	-	-	314 mm TL	526 mm TL	-	-	-
Kulaw 2013	Natural and artificial habitats; LA	Natural: 4 Artificial: 3	Natural: 6 Artificial: 8	Natural: 450 TL Artificial: 350 TL	Natural: 700 TL Artificial: 700 TL	Total mean: 219,258	Natural: 18 Artificial: 21 to 30	Total mean: 4,632,217
This Study	Natural and artificial habitats; LA	Natural: 4 Artificial: 4	Natural: 4 Artificial: 8	Natural: 401 TL Artificial: 501 TL	Natural: 401 TL Artificial: none	Natural: 704,563 Artificial: 41,878	Natural: 39 Artificial: 33	Natural: 26,323,179 Artificial: 1,369,334

VITA

Hilary Day Glenn was born in April 1985 in Auburn, California. She was raised as a fourth generation Loomis, California resident and graduated from Del Oro High School in 2003. From 2003 to 2007 she attended the University of California at Santa Cruz. In May 2007 she earned a Bachelor of Science in marine biology. After graduation, Hilary spent a year with Global Vision International in the Seychelles Islands teaching coral reef survey techniques to eco-tourists. In 2008 Hilary became a PADI dive instructor in Utila, Honduras. After this she split most of her time between teaching diving and working in the Alaskan Groundfish Fishery as an observer for Alaskan Observers Inc. In Alaska she gained first hand knowledge of commercial fishing and decided to continue her education. In June 2011 she joined Louisiana State University as Dr. James Cowan's graduate student. At LSU, she served two years as co-chair on the Education and Outreach Committee and one year as Treasure for the Coast and Environment Graduate Organization (CEGO). She also participated in the organization of the 2014 Graduate Student Symposium (GSS), hosted by CEGO. Hilary won an award for best student poster at the 2013 Southern Division American Fisheries Society Conference and the Ron Schmied Scholarship for the 2014 GCFI conference. She is currently a candidate for the degree of Master of Science in the Department of Oceanography and Coastal Sciences.