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Determination of Age and Size at Sexual Maturation of Yellowfin Tuna (*Thunnus albacares*) in the NW Atlantic Ocean

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DETERMINATION OF AGE AND SIZE AT SEXUAL MATURATION OF YELLOWFIN TUNA (*THUNNUS ALBACARES*) IN THE NW ATLANTIC OCEAN

BY

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ABSTRACT

This report examines sexual maturation of yellowfin tuna (*Thunnus albacares*; YFT) above 35° North in the western Atlantic Ocean. The majority of samples were collected near Oregon Inlet, North Carolina with additional samples from New England to determine if there is a difference in maturation with latitude. Maturation was determined by gross classification of gonads as well as histological examination.

Data were compiled based on sex and fish length to elucidate trends. Females collected at lower latitudes were found to have a lower GSI and gonad weight than those from higher latitudes. Males in both locations had a similar GSI and gonad weight based on the length of the fish. At higher latitudes, female fish were immature up to the largest fish collected (124 cm straight fork length (SFL)). Despite fish at lower latitudes having a lower gonadosomatic index (GSI) and gonad weight, beginning stages of oocyte development were present only in females ranging from 110-116 cm SFL in these locations. Overall, female fish were found to be in different stages of immaturity whereas a number of male fish were ready to spawn, suggesting that males may mature at a smaller size than females in both locations. In North Carolina, males ranging from 96-121 cm SFL were found mature, but only males ranging from 127-130 cm SFL were mature in New England. Results of this study are consistent with other studies that show size selectivity by angling gear and a delay in YFT maturation at higher latitudes.

Key words: *Thunnus albacares*, ovary, testis, sexual maturation

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DEDICATION

This work is dedicated to King Yellow, the one and only.

PREFACE

This work is written in manuscript format and adheres to the guidelines of the Graduate School of the University of Rhode Island. The manuscript is formatted in accordance with the guidelines set forth for publication in Fisheries Research.

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Determination of Age and Size at Sexual Maturation of Yellowfin Tuna (Thunnus Albacares) in the NW Atlantic Ocean

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CHAPTER 1

INTRODUCTION

Yellowfin tuna (*Thunnus albacares*; YFT) are a highly active pelagic fish found worldwide mainly between 45° N and 40° S in temperate and tropical waters (Collette and Nauen, 1983). YFT became a target commercial species in the 1950s, when a large-scale longline fishery began to proliferate, and remain a target species with the development of purse seines to capture fish near the surface. YFT annual catches in the Atlantic increased from 60,000 metric tons (MT) in the 1950s to a peak of 210,000 MT in the early 1990s. Since then, it has decreased to about 150,000 MT (Miyake et al., 2004). YFT are valuable in wild-caught fisheries and have generated interest as a potential aquaculture species. Critical to astute management of fisheries and aquaculture ventures is determination of when fish attain sexual maturity and spawn. Knowledge of sexual maturation is key in developing catch size limits for sustainable fishing and broodstock development for egg production for aquaculture ventures.

Research on the sexual maturation of YFT has been undertaken in the Pacific Ocean (Itano, 2000; Sun et al., 2005), Indian Ocean (Nootmorn et al., 2005; Marsac et al. 2006; Zhu et al., 2008; Zudaire et al., 2010;), and in the mid-Atlantic Ocean (Arocha et al., 2000, Arocha et al., 2001). The research conducted by Arocha and colleagues (2001) on YFT maturation in the central Western Atlantic examined a total of 133 ovaries in fish ranging from 120 to 173 cm straight fork length, and spanned

the large geographic range of 0° N to 55° N, with a focus on fish caught off the coast of Venezuela. Fish closer to the equator have a longer spawning season due to the water temperature and photoperiod. Information on maturation of YFT inhabiting latitudes above 35° N in the Western Atlantic is limited. The purpose of this study was to determine the size at which both wild tuna and captive broodstock in this region become sexually mature. This study also compared two methods to determine maturation stage– determination of gross maturation index (GMI) versus histological classification of gonads.

YFT spawning occurs throughout the year in the center of its geographical distribution, i.e., between 15° N and 15° S, but spawning may occur in other areas at specific times of the year (ICCAT, 1992). YFT in the Eastern Pacific have spawned at temperatures as low as 22° C, but most spawning activity takes place between 26° C and 30° C (Shaefer, 1998). Spawning is often inferred by the presence of YFT larvae or maturation stage of gonads. YFT larvae have been found in the Central and Western Pacific at temperatures above 24° C (Ueyanagi, 1969).

Major spawning grounds in the Atlantic are in the Gulf of Guinea (off West Africa; ICCAT, 1991) and the Gulf of Mexico (Arocha et al., 2000). Thousands of YFT tagged in the Northwest Atlantic have been recaptured in the Gulf of Guinea (Ortiz, 2001). Reproductively active females also have been found in other areas, including the western tropical Atlantic and the southwestern Caribbean off the coast of Venezuela (Arocha et al., 2000). YFT were found to spawn between February and November in the Western Central Atlantic (Arocha et al., 2000). Tagging studies of Atlantic YFT suggest a single stock with limited information on the extent of fish

spawning range (ICCAT, 1997). As YFT that are the size of sexually mature fish are caught in the Atlantic in areas outside of known spawning locations during the spring and summer months by recreational fishermen, spawning activity may be occurring. This study looks at YFT maturation in the Western North Atlantic to determine if spawning activity is occurring.

Many factors may affect maturation stage and rate of development. There is a large degree of variation among studies attempting to determine sexual maturation by examining captured fish (Table 1). Factors include location, gear type, method of determining, and whether both sexes were collected. One method that is often used to determine maturation is when 50% of fish reach maturity. To calculate the length at which 50% of sampled fish reach maturity, the sample population is split into 5 cm length classes and percentage of mature fish in each length class is calculated. Female YFT in the Western Pacific are 107.8 cm (Sun et al., 2005) when 50% of females are mature, and 92 cm in the Eastern Pacific (Schaefer, 1998). In the Eastern Indian Ocean, 50% of fish are mature at 95.4 cm for females and 99.6 cm mature for males (Nootmorn et al., 2005). Tuna, like many species of fish, may exhibit gear bias or avoidance as a result of vertical stratification of individuals in different reproductive states (Suzuki, 1994). Juvenile YFT are typically found near the surface and closer to the coastline, whereas larger fish may move offshore and remain near the surface waters (Miyake et al., 2004). Itano (2000) suggests a delay in maturation of Pacific YFT in higher latitudes compared to fish caught near the equator.

Many factors influence the growth and maturation of YFT. Nutrition, photoperiod, and water temperature may impact the rate at which YFT grow, and may

have an impact on sexual maturation or a hormonal response of the fish to begin maturation (Figure 1). Decreased water temperature leads to decreased feeding activity in captive YFT (Wexler et al., 2003). Water temperature also impacts the occurrence and timing of spawning activity, while an increase in food ration leads to an increase in egg production of female YFT (Margulies et al. 2005). Decreasing photoperiod in captive YFT also lead to cessation in spawning activity (Margulies et al., 2005). In the wild, water temperature has an impact on feed availability, growth rate, and sexual maturation (Lehodey and Leroy, 1999). Finally, time of year dictates photoperiod and water temperature for different locations, which may influence development of YFT. Some YFT spawn year-round and others spawn seasonally (Itano, 2000). These factors may also come into play with YFT in the NW Atlantic Ocean. In order for fish to spawn, both female and male sexually mature individuals need to meet under the right conditions.

YFT do not exhibit sexual dimorphism or external maturation characteristics, so the only means to determine sex and maturation stage at this time is to excise and examine the gonads. DNA sex-linked markers are currently available for Pacific bluefin but not yet for YFT (Agawa et al., 2014).

GMI classification is one way of determining sex and maturation stage of YFT tuna. This method of classification is based on the work by Schaefer (1987) and Nootmorn et al. (2005) and describes the gonads based on sight and physical characteristics of whole, uncut gonad for male, female, and immature fish. This method can be done in the field, as opposed to fixation and staining of tissue sections, but is not as accurate as histology (Schaefer, 2001).

Histological examination of gonad tissue is required to definitively determine specific maturation stages of YFT. Gross maturation indices may not be as effective for staging YFT, particularly females. In female YFT, the gonad diameter can be similar in both the vitellogenic phase and in postspawning, atretic fish (Schaefer, 2001). Fish are classified as immature when females have unyolked or early yolked oocytes and mature when advanced yolked oocytes or atresia are present. Males are classified as mature if there is histological evidence of the presence of sperm (Schaefer, 2001).

In the current report, fork length measurements and gonad samples were obtained from the recreational charter and private angling fishery. In North Carolina, samples were collected from charter fishing vessels at Oregon Inlet Fishing Center in Nags Head. In New England, all samples came from volunteer fishermen who measured the fish and excised the gonads to store on ice for the trip back to port. As maturation is linked to the size of the fish, the goal was to ascertain the size that fish begin to mature and at various stages of the process.

Information on the reproductive development of YFT is of value to fishery managers in the formulation of plans to protect stocks from overfishing and to facilitate maintenance of spawning populations. This study focused on a single species over a narrow geographic range, that is further north than other spawning studies in the equatorial Atlantic region. The size and weight at maturity of wild caught YFT may also be beneficial to YFT aquaculture research programs. Knowledge of these characteristics of tuna at different maturation stages would assist in collection and selection of potential broodstock that have attained or are approaching maturation.

This report also helps fill in the gaps of YFT tuna maturation characterization across the Atlantic Ocean. Of interest, previous investigations suggest that spawning size also varies with location (McPherson, 1991; Shaefer, 1998; Itano, 2000; Miyake et al., 2004; Nootmorn et al., 2005). Most studies on sexual maturation of tunas are limited due to the difficulty of collecting tissue from enough individuals to encompass fully immature to 100% mature fish during a single period of reproductive activity (Schaefer, 2001). The present report provides a comparison between sexual development and spawning of recreationally caught YFT in the equatorial and northern ranges of the species.

CHAPTER 2

MATERIALS AND METHODS

COLLECTION LOCATION

YFT were collected in two regions of the western North Atlantic Ocean off the coasts of Rhode Island and North Carolina. National Marine Fisheries Service regulations at the time of sampling prevented boats from retaining fish less than 27" curved fork length (HMS Recreational Compliance Guide, 2015). The largest number of fish were collected from Oregon Inlet, North Carolina (NC) (35.78°N, 75.53°W), the home of a fleet of charter fishing vessels. Collection in NC took place during June of 2014. Fish were also collected in southern New England (NE), from charter and recreational anglers fishing from Rhode Island, Massachusetts, and Connecticut (near 41.16°N, 71.58°W). Because there is a limit to the distance offshore these vessels can travel, as opposed to long liners or purse seiners, all fish in the present study were captured within 225km of shore. Fork length of the fish, water temperature, location of capture, and time of year were recorded. Sample collection in NE took place June to August of 2014. The number of YFT collected in NE was smaller than NC due to the absence of a centralized marina facility and willingness of anglers to participate.

The main difference between the two locations is the greater seasonality of water temperatures present in southern New England, where the water temperatures drop below that preferred by YFT in the winter months. YFT prefer temperatures greater than 17.5° C, but can tolerate temperatures as low as 11°C (Block et al., 1997).

Southern NE waters range from 3°C to 22°C annually, peaking in July and August (NOAA National Centers for Environmental Information, 2016). NC sea surface temperatures range from 15°C to 27°C, with temperatures above 25°C in June through September (NOAA National Centers for Environmental Information, 2016). YFT are caught recreationally in large numbers in June and July in NC, whereas they are caught in July and August in NE.

Samples from any YFT mortalities held at the University of Rhode Island (URI) were also compared to wild caught fish. These fish were collected at the locations in the Northeast Canyons (approximately 180-220 km offshore) described above and housed in the Blount Aquaculture Research Laboratory at the URI Narragansett Bay Campus.

CAPTURE AND PROCESSING

YFT were captured using standard recreational tuna angling gear, either by trolling or bait fishing. Fish collected by charter boats at the Oregon Inlet Fishing Center were brought intact on ice to the dock within 4 to 6 hours of capture. Upon return to the harbor, fish were immediately transferred to a fish processing facility located at the dock. Prior to processing, straight fork length (SFL) was measured to the nearest 0.2 cm using a flexible measuring tape and recorded. Curved fork length (CFL) was also measured. When possible, fish were weighed individually in 200-l barrels using a Toledo Model 700 floor scale with accuracy to 0.5 kg. Due to the nature of fish processing in this location, it was not possible to obtain weight for all fish.

In some cases, loins were removed from whole fish prior to the SFL being measured and gonads collected. After the loins were taken, fish were stored vertically in 200-l barrels in a room maintained at 4° C prior to measurements and collection of tissue. Storing them in this manner minimized bending or compression of the fish for accurate length measurement and preserved integrity of gonads. All samples were processed and tissue preserved within eight hours of capture.

To collect gonad tissue from intact fish, a ventral incision was made using a fillet knife to gain access to the peritoneal cavity. The gonads were excised and placed into a 1-l Ziploc bag marked with a unique identifier and stored on ice in a cooler.

Fish collected in NE were measured using the same methodology, where fish were brought to the dock whole. Curved fork length was recorded prior to the fish being gutted or decapitated; gonads were also collected at this time as above. Straight fork length was also measured. The fishermen were provided data sheets to record location of capture, sea surface temperature (if known), name, and the date. If the fishermen did not know the sea surface temperature, but were able to provide an estimation of the area fished, Satellite Imagery via the Rutgers University Coastal Ocean Observation Lab (Rutgers University) was used to estimate the sea surface temperature at point of capture. Gonads were weighed and sliced into thin sections of gonads placed into 10% neutral buffered formalin (NBF) as quickly as possible. NBF fixative was composed of 4.0 g monobasic sodium phosphate, 6.5 g dibasic sodium phosphate, 100 ml of 37% formaldehyde, and 900 ml of distilled water (Carson, 1992). In some instances, fishermen excised gonads and stored them on ice. These samples were collected immediately from fishermen for processing. Gonads were

weighed to the nearest 0.1 g using an A&D EK-2000 electronic balance for calculation of GSI. Visual inspection of gonads took place immediately after gonads were weighed and before subsections were cut for fixation.

Four tuna mortalities from the recirculating aquaculture system at the University of Rhode Island were measured and tissue collected as above. Three fish were collected prior to initiation of this study and maturation stage was determined histologically, since gross classification and/or gonad weight were not available. For the one additional mortality, gonads were visually classified and weighed.

For fish collected in all locations, weight of fish, gonadosomatic index (GSI), and the age of the fish were calculated. When use of a scale to weigh fish was not possible, the total weight of the fish (W_f) was estimated using the equation $W_f = a * SFL^b$ where a is1.886 x 10^{-5}, SFL is the straight fork length of the fish, and b is 3.0195 based on a sample of 6,752 YFT caught in the Indian Ocean (Marsac et al., 2006). GSI was calculated using the formula $GSI = W_g/W_f * 100$, where W_g is the weight of gonads in grams, and W_f is the total weight of the fish including gonads (Stequert et al., 2001). Age of fish was estimated using the equation $SFL = (245.541 * (1 - exp(-0.281 * (t - 0.0423))$, where t is time in years (Shuford et al., 2007). This growth equation provides an estimate, but does not take into account growth rate as water temperature-dependent.

Sex of fish was assessed by visual inspection and GMI when possible for samples from all locations. Undeveloped ovaries are round in cross-section and roll easily between fingers due to a lumen (Schaefer 2001). Developed ovaries are fusiform or spindle-shaped and circular in cross-section and tend to have a yellow hue

due to presence of yolk in the oocytes (Schaefer, 2001). Developed testes have a flattened appearance (in cross-section) and are usually white in color due to the presence of milt. Undeveloped testes are solid and cannot be rolled easily through the fingers (Schaefer, 2001). When illuminated, a longitudinal sperm duct is visible medially (Schaefer, 2001).

For GMI classification, gonads were staged using a visual index of 1 to 5 based on the work by Nootmorn et al., (2005) (Tables 2 and 3). The approach allows classification of maturation of male and female gonad samples using the same numerical scale of 1) immature, 2) early developing, 3) later developing, 4) mature, and 5) spawned. Classification characteristics include color, presence of a visible blood vessel, size, and shape of the gonad.

TISSUE FIXATION

Two sections of tissue were excised from the central section of the ovary or testis and sliced approximately 0.5 cm in thickness to facilitate penetration of formalin, perpendicular to the lumen. Depending on the diameter of the gonad, multiple 1-cm^2 sections were taken to obtain a sample of the entire cross section of the gonad. Gonad samples were placed into 15-ml conical tubes containing 10% NBF (Carson, 1992).

Tissue samples were fixed for at least 24 hours in 10% NBF and transferred to histology cassettes and 70% ethanol. Tissues were embedded and sectioned by a commercial laboratory (Mass Histology Services, Worcester, Massachusetts, USA); sections were stained with Harris' hematoxylin and eosin counterstain (Prophet,

1992). Tissue sections extended from the inner lumen to the outer wall of the gonad or about 1 cm in diameter outward if it was a large gonad.

DIGITAL IMAGING

Digital images of sections were captured using a Zeiss AxioImager M2 Imaging System and ZEN Digital Imaging Software (URI Genomics and Sequencing Center, Kingston, RI). Twelve fields at 20x magnification were photographed and the most advanced stage of development recorded; 20x magnification allowed for clear staging of gonads by presenting numerous gonad cells in a single field of view. Fields were chosen across the diameter of the slide to incorporate the range of development present. Further inspection of the images focused on the most advanced maturation stage present. At this magnification, most developed oocyte and cyst/duct diameters were measured using the scale in the ZEN Digital Imaging Software.

OVARIES

Classification of maturation of ovaries using histology was based on the work by Itano (2000) and Schaefer (2001) on YFT in Hawaiian waters and in the Western Tropical Pacific Ocean. Ten different maturity stages were used to define the degree of oocyte development, condition of oocytes, spawning stage, and degree of atresia (Table 4). Stages 1-3 include immature ovaries and stages 4-10 represent mature samples with advancing stages of spawning activity (Table 4).

Samples collected included ovaries at the primary growth phase of development. Thus, stages 1 and 2 of maturation (Table 4; Itano, 2000) have been

classified into more detailed developmental stages based on physical description and diameter of the oocytes (Table 5; Zudaire et al., 2013). For example, a stage 1 by Itano (2000), could then be a stage 1.1 or 1.2 by the descriptions from Zudaire (2013). A stage 2 by the descriptions from Itano (2000) would be a stage 2.1 or 2.2 by Zudaire (2013). As each oocyte develops, it increases in diameter due to the development of yolk plates and the uptake of fluids. Mature oocytes can be up to 750 μm in diameter.

TESTES

Maturation stage of the testes using histology was based on the degree of spermatogenesis within the germ cells using the system developed by Schaefer (2001). All germ cells within a cyst were approximately at the same level of development. Stages of maturation of sperm were classified as primary spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa (Table 6).

To further characterize testis, the diameter of the largest cyst or duct was measured and presence or absence of milt in the lumen of the testis was recorded. The percentage of the testis comprised of cysts and ducts was estimated across the area of the sample. As testes develop, the cysts lyse and form ducts which move milt toward the lumen. A large number of cysts suggest testes are less developed (Schaefer, 2001).

CHAPTER 3

RESULTS

NC SAMPLES

A total of 385 samples were collected from Oregon Inlet from June 15-17, 2014. Fish were caught less than 65 km offshore where sea surface temperature was approximately 26° C. All samples were visually classified as male, female or unknown and given a GMI classification of 1-5.

Of the samples from Oregon Inlet, weight was measured for 29 fish (Figure 2a). The heaviest fish was a male weighing 29.0 kg, and the lightest was a female weighing 5.9 kg. The equation relating SFL in centimeters to whole body weight (W_f) in kilograms for male fish is W_f=0.4484(SFL)-25.6 and for female fish is W_f=0.4603(SFL)-27.7. This equation was not used to calculate GSI due to the low number of samples (n=19). Fish whose gender was not able to be determined were not included. Female fish had a higher gonad weight per total body weight than male fish (Figure 2b).

Based on GMI classification, 83 fish were male, 205 were female, and 97 were unknown (Table 7). Maturation of female and male fish was considered at GMI classification stage 4. Eight of the male fish and 63 of the female fish were found to be at stage 4 (Table 7). No fish were found to be at stage 5, i.e., spawning or post-spawn. In stage 4 females the gonad is pale orange and rounded, when the oocytes are ready to be released from the follicle layer and ovulated. The smallest fish to reach this stage was 71 cm or approximately 1.3 years of age (Figure 3c). In males, stage 4 was

characterized by testis which contain milt and are white or reddish in color. The smallest male to reach stage 4 was 96 cm long, or 1.8 years of age (Figure 3d). All samples where sex could not be determined by GMI classification were at GMI stage 1.

In total, 105 samples from NC fish were processed for histology. The initial batch of samples for histology included 35 females, 35 males, and 35 unknown sex based on GMI classification. Histological examination showed that 45 were female, 58 were male, and 2 could not be classified.

The 45 females ranged in length from 65 cm to 116 cm, which equates to an approximate age of 1.14 to 2.32 years (Table 8; Figure 4c). The GSI for fish determined to be female by histological classification ranged from 0.054 to 1.062 (Figure 5c). Overall gonad weight ranged from 3.5g to 123.2g. As SFL of NC female YFT increases, gonad weight does as well (Figure 6c). For NC samples, gonad weight and GSI are closely related.

Based on histology, ovary samples were more immature than expected given the size of the fish. As oocytes develop, they increase in diameter. All female samples were stage 1 out of 10 except 5 that were stage 2 (Table 4). Because all female samples were immature, a more precise staging was used to classify immature oocytes into more specific categories (Table 5). The more precise staging took into account degree of development and oocyte diameter. Of the samples that were immature, twenty-two samples were stage 1.1 (perinucleolar), 18 samples were stage 1.2 (chromatin), 4 were stage 2.1 (cortical alveoli), and one was stage 2.2 (early stage of

vitellogenic, vtg 1) (Table 9; Figure 7c). No fully yolked oocytes were present, suggesting no female fish were ready to spawn.

The diameter of the largest oocytes ranged from a maximum single oocyte diameter of 40 µm in one female to 270 µm in another (Figure 8). The ovary with the smallest oocytes was classified stage 1.1 and the ovary with the largest oocytes was stage 2.2 (Table 5). This suggests that the most developed ovaries were still in the primary growth phase and just beginning to develop yolk vesicles.

To be considered approaching maturation, females had to be at above a stage one, i.e., at stage two (Table 4) or at stage 2.1 or 2.2 the early stage of vitellogenic (Table 5). Five of the females were classified as beginning to mature (Table 9) or 11.1% of the females collected. The smallest female that reached stage 2 was 110 cm. Similar SFL fish do not all mature at the same rate and therefore may be in different maturation stages.

The 58 males as determined by histology ranged in length from 62 cm to 121 cm, which is an estimated age span of 1.1 to 2.5 years (Table 10; Figure 4d). The two unknown samples were 67 cm and 102 cm SFL, which is an estimated age of 1.2 years and 1.9 years, respectively. The GSI for these males ranged from 0.027 to 1.022 (Figure 5d) with a gonad weight of 1.1g to 114.5g. As SFL of male YFT increases, gonad weight does as well (Figure 6d).

Overall, there were fourteen males at stage 5 (24%), the most mature stage present with spermatozoa present in the testes (Table 6). The average size of the males that attained maturity was 109.6 cm, with a range of 96 cm to 121 cm. The average size of males that were not mature was 92.8 cm, with a range of 62 cm to 113

cm. Fish about 95 cm to 118cm SFL could have been any maturation stage, indicating a high range of variance in maturation for a certain SFL of fish. If more male samples were collected and found mature, an attempt to assess length when 50% of the fish was mature would have been assessed. Seven males were at stage 4, four at stage 3, five at stage 2, and twenty-eight at stage 1 (Table 11; Figure 7d). Of the testes from 58 males, thirty-nine were comprised solely of cysts, or an early immature stage. Only three of the 58 testes samples were comprised of 100% ducts, i.e., all cysts had ruptured and the testes were mature. Two of the samples processed for histological analysis could not be sexed and staged due to the slide quality.

NE SAMPLES

Of the 46 samples from NE, thirty were collected from an offshore fishing competition (Block Island Tri-State Tournament Shootout; August 4-6, 2014) within 160 km of Block Island, Rhode Island. The sea surface temperature where the fish were caught was approximately 25 °C (Rutgers University). The remaining 16 were collected by recreational fishermen either on July 12 or 18 approximately 100 km to 120 km to the Southeast of Block Island, Rhode Island in 23 °C water. All NE samples were grouped together due to proximity of date of capture and sea surface temperature. Based on GMI classification, 10 were male, 26 were female, and 10 were unknown.

Females and males were considered mature at a GMI classification of stage 4 when gonads are in active spawning condition. Based on this classification, the

smallest female fish at stage 4 was 82 cm (Figure 3a); a single male was categorized as stage 4 (130 cm SFL; Figure 3b).

Thirty samples were assessed by histology including 10 identified as males from gross classification, 10 identified as females, and 10 of unknown sex. These samples were chosen to reflect all the male and unknown sex fish, and ten females that included the fish with the maximum straight fork length, minimum straight fork length, mean fork length, one standard deviation above and below the mean. The samples also included the minimum gonad weight, maximum gonad weight, mean, and one standard deviation above and below. The sex of all samples could be identified by histological examination with 11 determined to be females, and 19 males.

The eleven females ranged from 69 cm to 124 cm SFL, an estimated age range of 1.2 to 2.5 years (Table 12a; Figure 4a). GSI ranged from 0.22 to 6.28 (Figure 5a), and gonad weight from 4.1g to 73.8g. SFL and gonad weight does not have a strong relation in NE female YFT (Figure 6a). Males ranged from 78 cm to 130 cm SFL, with an estimated age range of 1.4 to 2.7 years (Table 13a; Figure 4b). GSI ranged from 0.20 to 0.85 (Figure 5b) and gonad weight from 2.3g to 38.1g. For NE male YFT, as SFL increases, gonad weight does as well (Figure 6b). The GSI (r square= 0.13) and gonad weight (r square= 0.00) for females from NE was less related to SFL than it was for GSI (r square= 0.47) and gonad weight (r square= 0.49) for females from NC.

Histological examination showed that six ovaries were in the chromatin nuclear stage and 5 in the perinucleolar, with none at more developed stages (Table

12b; Figure 7a). The diameter of the largest oocytes measured for each fish ranged from 55 μm to 100 μm. The ovary with the smallest oocytes was at stage 1.1 and the ovary with the largest oocytes was stage 1.2. Based on histology, none of the female samples from NE were mature. Females in NE were less developed compared to NC, where 11% of females were beginning to mature. Both stage 1.1 (chromatin nuclear) and 1.2 (perinucleolar) reflect stages of primary growth of the ovary, rather than maturation.

The maturation stage of male gonads was also determined by histological examination. Two males were at the most advanced stage (5; spermatozoa), none at stage 4 (spermatids), five at stage 3 (secondary spermatocytes), eight at stage 2 (primary spermatocytes), and four at stage 1 (primary spermatogonia) (Table 13b; Figure 7b). Out of 19 male samples, 15 samples were comprised of 100% cysts (suggesting immaturity), two had both cysts and ducts present, and two samples with 100% ducts. The smallest cysts were 50 μm and at stage 1, and the largest cysts were 150 μm in diameter and at stage 5.

Males were considered mature when they were at a histological classification of stage 5, indicating mature spermatozoa in the testes. Of the 19 samples, two were stage 5, or 11% of the males from New England. The mature males were 127 cm and 130 cm SFL. Males in NE were less developed compared to NC, where 24% of males were mature.

RI SAMPLES

Samples were collected from four YFT housed at the University of Rhode Island. The sex could not be determined by visual classification. Based on histological classification, three were unknown or undeveloped and one was a stage 5 male. The three unknown fish ranged in SFL from 78 cm to 98 cm, and a weight range of 6.92 kg to 13.28 kg. The mature male was 122 cm SFL and 38.44 kg. Gonad weights were not available.

CHAPTER 4

DISCUSSION

This report is the first attempt toward characterizing the initiation of sexual maturation for recreationally caught male and female YFT tuna in the Western Atlantic Ocean outside of the tropics, in the area above 35° North. As YFT are migratory, this study aimed to determine if reproductively mature individuals could be found during the summer months off the coast of NC and NE. The majority of research studies on YFT maturation focus on longline or purse seine caught fish in the tropical oceans (Arocha et al., 2000; Itano, 2000; Sun et al., 2005; Zudaire et al., 2010). Evidence suggests tuna exhibit gear bias and vertical stratification of individuals of the same reproductive stage of development (Suzuki, 1994; Davis et al., 1998) which may have impacted the samples included in this research. The present study begins to characterize nearshore fish caught using recreational fishing methods rather than examining more open ocean populations and commercial fishing techniques.

The range of gonad weights for both male and female fish was larger in NC than in NE. In NC, females had gonad weights from 3.5 g to 123.2 g and males from 1.1 g to 114.5 g. Females in NE had gonad weights from 4.1 g to 73.8 g and males from 2.3 g to 38.1 g. The GSI for females and males in NC was similar, with a range of 0.05 to 1.06 for females and 0.03 to 1.02 for males. In NE, females (0.22 to 6.28) had larger GSIs than males (0.20 to 0.85). In NE the seasonality present may force fish to physiologically decide between putting energy toward growth or sexual

development. In NC, where the water temperature and photoperiod are more stable, the GSI and gonad weight appeared to increase relative to SFL at a more predictable rate. It is interesting that female fish in both locations had higher GSIs and gonad weights than male fish as the females were just beginning to mature but the males were at higher maturation stages. Higher GSI in females has been evidenced in other species, including dolphinfish (Castro et al. 1999), tilapia (Mahomoud et al. 2011), and marine catfishes (Gomes and Araujo, 2004). Two of the male fish in NE were mature with gonad weights of only 11.9 g and 38.1 g.

In the present study, microscopic examination proved to be a much more accurate method for maturity staging than GMI visual classification. Microscopic examination was used to definitively identify samples that were first classified visually. For fish caught in NC, 78% of the fish that had been grossly identified as female and 48% of male fish were corroborated by histological examination. Three percent of samples could not be identified using the gross maturation index. For fish caught in NE, visual classification was accurate for 63.6% of female samples, and 47.3% of male samples. The remaining percentages of fish samples that were incorrectly identified visually were either the opposite sex or could not be identified by histology. All samples from NE were able to be sexed using histology. In contrast, in NC 98% of samples were correctly identified by histological examination, where 43% were definitively identified as females and 55% as males. Only 2% of samples could not be identified. In NE, 100% of samples were able to be sexed and staged using histological examination, with 37% females and 63% males. Histological examination is a more accurate and definitive, though time consuming, method of

sexual maturation staging than gross classification of gonads for species that exhibit no outward sexual dimorphism. The use of histological examination to assess maturation stage of gonads is the most precise staging method (Schaefer, 1998).

This study supports previous research that suggests fish at higher latitudes may mature at larger sizes as compared to fish closer to the equator (Itano, 2000). Tagging studies of Atlantic YFT suggest a single stock (ICCAT, 1997) so the fish may be travelling through different latitudes and maturing at different rates based on water temperature and food availability. Higher latitudes have a lower annual average sea surface temperature. While the theory that there is a single stock of YFT in the Atlantic is currently accepted, future research should be done on genetics of YFT in this area, as recent genetic work shows there are at least three genetically discrete populations of YFT in the Pacific (Grewe et al., 2015).

Results of this study show a difference in the timing of maturation of female fish as compared to male fish in the same location. This has been witnessed in other species, such as salmonids (Barson et al., 2015) and tilapia (Bhatta et al., 2013). A difference in timing of maturation of females and males has also been evidenced in YFT the Eastern Indian Ocean (Nootmorn et al., 2005) and the Eastern Pacific (Schaefer, 1998). In the Eastern Indian Ocean, YFT caught by surface longlines were found to be first mature at 109.69 cm for females and 104.95 cm for males. In the Eastern Pacific, 50% of female YFT were mature at 92 cm, and 50% of males were mature at 69 cm. In NC, the average size of fully mature males was 109.6 cm SFL and female fish were beginning early maturation stages at 110 cm FL. Males mature at a younger age and size than females. Females have more stages of development to go

through including yolk development and hydration of oocytes before they attain full maturation with oocytes ready for ovulation. Further north, in NE, male fish were found to be mature at 127 cm FL and none of the female fish had reached the early maturation stage.

Life stage sexual dimorphism has been observed in YFT in the eastern Pacific where Wild (1986) found that initially females are larger than males of the same age, growth curves cross around age 2 (where fish are approximately 95 cm FL) and then males are larger than females of the same age. Further investigation showed growth rate of males is higher than females (Wild, 1994). This may be due to the amount of energy required for oocyte production and ovulation. In the Western Atlantic, with appropriate seasonal temperature and light requirements, tuna spawn every 1.47-3.35 days and produce 54.2 oocytes per gram of body weight per spawn event (Arocha et al., 2000).

The results from the current study are consistent with a previous year-long study on immature female YFT caught in the tropical Western Atlantic Ocean which showed actively spawning females to be larger than those sampled in this study. Closest in geographic location to this study, Arocha et al. (2000) examined morphometric data of 42,717 YFT in the western Central Atlantic, between 5 degrees North and 35 degrees North. They found that most of the actively spawning female YFT were between 130 cm and 150 cm FL, with only a small fraction of reproductively active fish between 100 cm and 110 cm FL. Based on 106 ovaries analyzed, Arocha et al., (2000) observed that spawning activity takes place in the Gulf of Mexico and southeastern Caribbean Sea from May to August. In the present study,

mature males were found in NC in June and NE in July and August. Mature males in these areas are not going to reproduce even if they are able if the females are not ready to spawn. Alternatively, the males may move around to locate suitable mates. No females in this study were fully mature. Females in NC were in the early stages of maturing at a minimum length of 110 cm. There was a distinct lack of larger YFT collected for this study, suggesting that larger fish remain further offshore, in spawning areas, or were not selected based on the gear type used.

Size at first maturity for males and females in this study is greater than that for fish caught in the central and western Indian Ocean. Zudaire et al. (2010) found female fish caught by purse seines in the equatorial area of the Indian Ocean first mature at 77.8 cm FL. More commonly reported than size at first maturity is size that 50% of the sample population reaches maturity. Itano (2000) focused on females caught by purse seine, longline, and handline in the central and western Pacific Ocean and found that fish were 50% mature when they were 107 to 120 cm in length. Sun et al. (2005) found that female YFT caught by longline were 107.77 cm in length when they are 50% mature in the western Pacific Ocean, or 2.4 years old. In the eastern Pacific Ocean, Schaefer (1998) found that females were 92.1 cm when 50% of the fish were mature. If more larger and reproductively mature fish were obtained during sample collection for this study, an attempt at determination of length at 50% maturity would have been attempted.

Findings of this study are consistent with previous work that shows size selectivity of fishing gear. Fish caught in NC ranged from 62 cm to 121 cm with an average of 96 cm and fish caught in NE were 69 cm to 132 cm with an average of 105

cm. This is in the mid-range of YFT sizes caught by purse seine and by longlines. Arocha et al. (2000) found that fish caught by purse seine ranged from 35 to 145 cm but were predominantly 60 to 75 cm, whereas the longline caught fish ranged from 40 cm to 140 cm but are predominantly 135 to 140 cm FL. In the Japanese YFT fishery, purse seines are used to capture fish smaller than 70 cm and longline gear is used to harvest fish larger than 90 cm (Suzuki, 1988). Actively feeding yellowfin schools are often made up of reproductively active fish; these tuna aggregations where spawning occurs daily or near-daily influences the vulnerability of the tuna to purse seine gear (Itano, 2000). Mature but reproductively inactive fish are more likely to be captured with deep-set longline gear (Itano, 2000).

The results of this study are of value to fisheries managers. The size limit for retention of YFT in both commercial and recreational fisheries in the US Atlantic is 68 cm (27 inches) CFL. SFL and CFL are closely related. For bluefin tuna, SFL= 0.972*CFL (r square=0.999, $p<0.001$, n=1,308; Salz et al., 2007). Fish at this size were found to be immature in both locations. If fish are continuously caught at this small size, these fish are unable to spawn and replace themselves in the wild prior to being caught. The largest female fish from NC, at 116 cm, was just beginning the yolk development stage. None of the female fish collected in NC or NE were ready to spawn so a larger minimum size limit is recommended.

Additionally, knowledge about sexual maturation of YFT would be beneficial to aquaculturists for developing methods to culture large pelagic species. Three of the four samples taken from broodstock mortalities showed immature gonads, at fork lengths up to 98 cm. Capturing broodstock that are already mature can decrease

holding times for fish to reach maturity. Tuna aquaculture has increased globally, with tuna farming and ranching, both inshore and offshore in net pens. YFT aquaculture is gaining popularity, with research centers in Panama, Hawaii, Indonesia, Australia, and Rhode Island. YFT are asynchronous spawners, producing up to several million eggs daily given the right temperature and photoperiod.

The Achotines Lab in Panama has been successful in spawning YFT tuna for a period of nine months when the temperature and light were manipulated (Margulies et al., 2007). These broodstock were 51 cm to 78 cm in length, with an average of 62 cm FL and were collected from waters near the laboratory in Panama. The YFT collected for broodstock are a Pacific strain of YFT found close to the equator. The fish at Achotines attained maturity at a smaller size than the fish collected in this study. The smallest male collected in NC that attained maturity was 96 cm, with an average length of 109.6 cm for mature males. None of the females were mature in this study, up to the largest female at 116 cm. The results from the University of Rhode Island broodstock samples show a delay in maturation as compared to wild caught fish, despite being in a tank with manipulatable photoperiod and seawater temperature. The gonads were undeveloped in three out of the four broodstock fish sampled, up to 98 cm. A 122 cm long fish was a fully mature male. It is possible that the broodstock maturation in the tank was inhibited by stress response to captivity or a lack of social cues. Stress response has been observed to impair many functions of fish species (Harper et al., 2009) including oocyte maturation in common carp (Wang et al., 2008) and decreased GSI and gametogenesis (Thomas et al., 2007). As three of the four

samples were unable to be sexed, the gender ratio of broodstock in the tank could not be determined.

There is evidence that YFT spawn in the Gulf of Mexico, Caribbean, and Gulf of Guinea. Arocha et al. (2001) found YFT spawn in the Gulf of Mexico between May and August and in the southeastern Caribbean Sea between July and September. Only a small fraction of the spawning females were less than 130 cm FL, but overall the spawning females were smaller in the Gulf of Mexico than in the Southeastern Caribbean. Arocha et al. (2001) believed fish spawned in the Southeastern Caribbean contribute to the YFT populations off the coast of Venezuela and Guyana, whereas the fish spawned in the Gulf of Mexico contribute to the fish off of the United States and Mexico. There is minimal exchange between the two spawning locations, which may support multiple distinct populations of YFT in the Atlantic. Adult YFT also spawn in the Gulf of Guinea from November to March, with retention of larvae and juveniles for approximately one year (Richards, 1969). After that, they move south to Angola, and then north to warmer water. YFT older than two years of age then travel to the central tropical Atlantic, but they do return to the Gulf of Guinea in the warm months to spawn. It appears likely that fish collected for the present study were spawned in the Gulf of Mexico, as it is the nearest spawning location to the region of collection.

The findings of this research may be translatable to other species, including Atlantic bluefin tuna (*T. thynnus)* and Pacific bluefin tuna (*T. orientalis*), where the commercial value can be as high as thousands of dollars per fish. YFT may also serve as a model species because they can reach maturity at smaller sizes than other tuna species; 70 to 80 cm in the equatorial Pacific, with 50% of the population mature at

105 cm (Lehodey and Leroy, 1999). In this study, both male and female fish were found to mature at larger sizes than in the equatorial Pacific, and at larger sizes the further from the equator they were collected. In contrast, populations of bluefin tuna caught in the eastern Atlantic Ocean are 135 cm or 3 years of age when 50% mature, and can take 4 to 5 years for the entire population to reach maturity (Corriero et al., 2005). Atlantic bluefin can live upwards of 35 years (Corriero et al., 2003). Bigeye (*Thunnus obesus*) caught in the tropical Atlantic also mature at a larger size, as they attain first maturity at 104 cm for females and 108 cm for males (Guoping et al., 2011). YFT may be a more ideal tuna species for captive broodstock type scenarios.

One of the limitations of this study is that only females at early stages of maturation were collected. Broadening the study to incorporate larger sized YFT may have helped better characterize maturation in female fish. Tagging fish caught recreationally in NC and NE to determine migration patterns as fish grow and mature may also provide information about size selectivity and maturation stage selectivity of fishing gear. At present, there is one study which shows sportfish, longline, and purse-seine caught fish tagged in the western Atlantic recaptured near the Gulf of Guinea (Ortiz, 2001). It would be of interest to tag small males and females and track their whereabouts throughout their maturation rather than simply a tagged location and recapture location. If location and sea surface temperature could be obtained throughout the summer months when most YFT spawn, alternative spawning grounds might be detected. Tagging studies in the Gulf of Mexico show YFT dive deeper during the day and stay near the surface at night, but the short duration of the study (80 days) did not show lateral movement outside of a bias against areas that were more

than 6°C cooler than surface water temperature (Weng et al., 2009). Tagging studies in the northeastern Pacific show YFT remained within 1,445 km of their tagging location even after 1,161 days had passed (Schaefer et al., 2007).

To conclude, YFT in the western North Atlantic mature at a different rate as compared to YFT caught in tropical waters, with more mature fish at lower latitudes. With YFT being migratory, it is possible that YFT in the western North Atlantic may move south or offshore as they become sexually mature. Males were found to mature at smaller sizes than females in NC and NE. More research should be on broodstock YFT maturation due to the low sample size included in this study. A future research direction could be to tag recreationally caught fish to see where they aggregate when they are reproductively mature, or to determine if there is a place further north in the Atlantic Ocean than the Gulf of Mexico where spawning activity occurs. Genetic testing would also provide insight as to how distinct the stocks of YFT in the Atlantic are- do they have predictable migration patterns, remain dissociated, or do they intermingle? By further developing the current understanding of this incredible species, YFT can continue to be researched and harvested sustainably for conservation efforts, captivity for aquaculture, and consumption worldwide.

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Table 1: Yellowfin tuna maturation for locations in different ocean basins with metric and capture method
Footnotes: a) 50% of females reach maturity, b) 50% of sample reached maturity (separated by sex), c) average size of mature fish

Ocean Basin	Location	Size at Maturity		Capture method and sample size (n)	Source
		Females	Males		
Pacific	Eastern Pacific	92cm[a]	69cm[a]	Purse seine N= 7121 females and 7077 males	Schaefer, 1998
	Western Pacific near Taiwan	107.8cm[a]		Longliners n= 1613	Sun et al., 2005
	Philippines and Indonesia	98.1cm[a]		Hand line n=3630	Itano, 2000
	Equatorial Western Pacific	107.9cm[a]		Purse seine, Longliner n= 3935	Itano, 2000
	Near Hawaii	112.5cm[a]		Longliner, handline, troll caught fish n= 899	Itano, 2000
Indian	Western and central Indian Ocean	77.8cm[a]		Purse seine n= 1561	Zudaire et al., 2010
	Seychelles	104cm[a]		Purse seine n= 1088	Marsac et al., 2006
	West-Central Indian Ocean	114 cm[b]	120cm[b]	Longliners n= 1023	Guoping et al., 2008
	Eastern Indian Ocean	95.4cm[b]	99.6cm[b]	Longliners n= 495	Nootmorn et al., 2005
Atlantic	Western Atlantic	Immature up to 116cm	109.6cm[c]	Recreational methods (troll, baitfishing) n= 385	current study

Table 2: Gross classification of maturity stages of yellowfin tuna ovaries by Nootmorn et al. (2005) that follow descriptions by Schaefer (1987)

Stage	Classification	Description
Stage 1	Immature	Thin hollow tubes 3-4 mm diameter and color translucent/white.
Stage 2	Early Developing, recover spent	Oocytes visible on inner ovary wall. Blood vessel visible distinctly on external of ovary wall. Color pale reddish/orange.
Stage 3	Later Developing	Ovary and oocytes develop, oocytes shape not round and tightly attached. Blood vessel visible less than previous stage. Color pale orange.
Stage 4	Mature	Ovary is well developed. Oocytes slip off inner of ovary wall, shape rounded and translucent area surround opaque oocytes. Color pale orange/ yellow.
Stage 5	Spawned	Characteristic ovary softened, deflated and flaccid. The rest of oocytes found in ovary. Color dark orange/yellow.

Table 3: Gross classification of maturity stages of yellowfin tuna testis by Nootmorn et al. (2005) that follow descriptions by Schaefer (1987)

Stage	Classification	Description
Stage 1	Immature	Thin hollow tubes 3-4 mm diameter and color translucent/white.
Stage 2	Early developing, recover spent	Testis tubes develop and blood vessel visible in the tube. Spermatogonia and spermatocytes present and mitotically divide in nests. Color pale white/reddish.
Stage 3	Late developing	Testis tubes well developed and blood vessel visible less than previous stage. All stages of sperm development, ripe sperm becoming abundant in cysts and lobule lumen but not in ducts. Color white/ reddish.
Stage 4	Mature	Gonad full of sperm, packed with ripe sperm in lobules and ducts. Color white/ reddish.
Stage 5	Spawned	Gonad softened, deflated and flaccid. Color dark white.

Table 4: Female yellowfin maturation stage based on histological examination of oocyte condition and evidence of atresia, including characteristics of stages classified as mature and immature (Itano, 2000)

Stage	Maturity classification	Oocyte Condition	Atresia	Comments
1	Immature	Majority of oocytes in late diplotene or early perinucleus stage	No atresia	Densely packed oocytes darkly stained with Hematoxylin
2	Immature	Mix of early or late perinucleus stage oocytes. No yolk granules present	No atresia or minor areas of unyolked oocytes	Early developing stage
3	Immature	Partially yolked	No atresia or minor areas of unyolked oocytes	Red staining yolk granules or globules evident from cell periphery inward to within 3/4 of distance to perinuclear zone
4	Mature	May be unyolked or partially yolked	Atresia of fully yolked oocytes evident	Considered to have reached a fully yolked and potentially reproductive state but regressed to a reproductively inactive state
5	Mature	Fully yolked oocytes present but no post ovulatory follicles observed	Zero or less than 50% atresia of fully yolked oocytes	A mature, potentially reproductive fish
6	Mature	Fully yolked oocytes present. Oocytes may be in migratory nucleus or hydrated condition and/or post ovulatory follicles present	Less than 50% atresia of fully yolked oocytes, generally zero or minor atresia	An actively spawning fish with zero or minor atresia. A typical, actively reproductive and spawning fish.

7	Mature	Fully yolked oocytes present. Oocytes may be in migratory nucleus or hydrated condition and/or post ovulatory follicles present.	Atresia of 50% or more of fully yolked oocytes	An actively spawning fish with significant atresia
8	Mature	Some fully yolked oocytes present but none in migratory nucleus or hydrated condition. No postovulatory follicles present	Atresia of 50% or more of fully yolked oocytes	A potentially reproductive fish with significant atresia
9	Mature	No fully yolked oocytes but atresia of fully yolked oocytes evident	100% atresia of fully yolked oocytes	A mature fish in non-spawning phase
10	Mature	No fully yolked oocytes present. Oocytes resemble Stage 1 or 2.	Advanced atresia of oocytes	A mature fish in advanced atretic, post spawning phase

Table 5: Oocyte descriptors and oocytes diameters using the table by Zudaire et al. (2013)

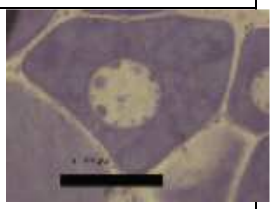
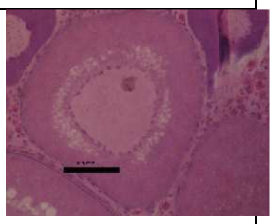
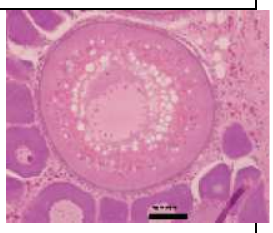
Stage Number	Oocyte development stage	Characteristic	Oocyte diameter (µm)	Image
1.1	Primary growth: chromatin nuclear	Oocyte is surrounded by squamous follicle cells. Nucleus is large and located near the center with a single large nucleolus, surrounded by cytoplasm.	45-75	
1.2	Primary growth: perinucleolar	Nucleus increases in size and numerous nucleoli appear at the periphery. Balbiani bodies migrate from nucleus to cytoplasm. At the end of this stage, some vacuoles appear in cytoplasm and chorion precursor material accumulates in patches.	75-120	
2.1	Cortical alveoli formation	Spherical vesicles start to appear at periphery of cytoplasm. They increase in size and number, forming rows which give rise to cortical alveoli. Oil droplets begin to accumulate in cytoplasm. Chorion and follicle layers are now apparent.	120-200	
2.2	Vitellogenic	This stage characterized by appearance of yolk vesicles in cytoplasm. Separation of the chorion into two different layers: called the inner and outer zona radiata. In vtg1, oil droplets occupy more cytoplasmic area than yolk granules.	200-310	

Table 6: Testes descriptors based on work by and images from Schaefer (2001)

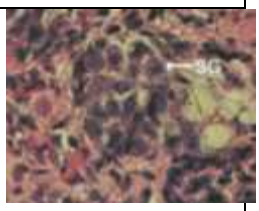
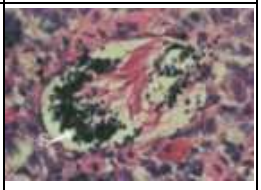
Stage	Classifi-cation	Most developed stage present	Description of stage	Image
1	Immature	Primary spermatogonia	Largest cells, 12-16 µm. Cells found near periphery of testis, with a single prominent nucleus and basophilic stain. Lighter colored than secondary spermatogonia. Found in cysts.	
2	Immature	Primary spermatocytes	Develop from primary spermatogonia by mitotic division. Cells are oval or spherical and are smaller than spermatogonia with a diameter of 8-12 µm. No nuclear membrane, dark chromatin material occupies cell.	
3	Immature	Secondary spermatocytes	Smaller, spherical cells with diameter of 4-7 µm. Chromatin is clumped together. Cells occur in groups and are very basophilic.	
4	Immature	Spermatids	Produced by mitotic division of secondary spermatocytes. Strongly basophilic spherical cells with diameter of 2-4 µm. As they mature they become smaller and remain in dense clusters.	
5	Mature	Spermatozoa	Spermatozoa are smallest of all germ types found in testis, with a diameter of 1-2 µm excluding tail.	

Table 7: Gross classification of maturation stage of gonads of yellowfin tuna collected in Oregon Inlet, NC separated by sex

NC Gross Classification		Unknown	Male	Female	Totals:
Stage 1	Immature	97	0	0	97
Stage 2	Early Developing	0	54	55	109
Stage 3	Later Developing	0	21	87	108
Stage 4	Mature	0	8	63	71
Stage 5	Spawned	0	0	0	0
	Totals:	97	83	205	385

Table 8: Straight fork length, age, GSI and gonad weight of female yellowfin tuna collected in NC

NC Females	Straight Fork Length (cm)	Age (years)	GSI (based on calculated weight)	Gonad Weight (g)
Minimum	65	1.14	0.054	3.5
Maximum	116	2.32	1.062	123.2
Mean	95	1.80	0.28	28.48
Standard Deviation	14	0.33	0.22	26.25
Number of samples	45			

Table 9: Histological classification of maturation stage of female yellowfin tuna collected in NC

Stage		Number	Range of Straight Fork Lengths of Fish (cm)	Oocyte size range (µm)
1.1	Chromatin nuclear	22	65-108	40 - 70
1.2	Perinucleolar	18	82-112	75-120
2.1	Cortical alveoli	4	110-116	125-200
2.2	Vtg 1	1	110	275

Table 10: Straight fork length, age, GSI, and gonad weight of male yellowfin tuna collected from NC

NC Males	Straight Fork Length (cm)	Age (years	GSI (based on calculated weight)	Gonad Weight (g)
Minimum	62	1.08	0.027	1.1
Maximum	121	2.46	1.022	114.5
Mean	97	1.84	0.113	11.9
Standard Deviation	14	0.33	0.155	17.63
Number of samples	58			

Table 11: Histological classification of maturation stage of male yellowfin tuna collected in NC

Stage		Number	Range of Straight Fork Lengths of Fish (cm)	Diameter of largest cyst or duct range (µm)
1	Primary spermatogonia	28	62-109	30-70
2	Primary spermatocytes	5	65-106	50-55
3	Secondary spermatocytes	4	87-107	45-60
4	Spermatids	7	81-113	60-110
5	Spermatozoa	14	96-121	70-200

Table 12: A) Straight fork length, age, GSI, and gonad weight and B) histological classification of maturation stage of female yellowfin tuna collected in NE

A

NE Females (based on histological classification)	Straight Fork Length (cm)	Age (years)	GSI (based on calculated weight)	Gonad Weight (g)
Minimum	69	1.21	0.22	4.1
Maximum	124	2.54	6.28	73.8
Mean	98	1.89	1.87	32.15
Standard Deviation	20	0.47	2.01	23.71
Number of samples	11			

B

Stage		Number	Range of Straight Fork Lengths of Fish (cm)	Oocyte size range (µm)
1.1	Chromatin nuclear	6	69-117	55-85
1.2	Perinucleolar	5	82-124	75-100

Table 13: A) Straight fork length, age, GSI, and gonad weight and B) histological classification of maturation stage of male yellowfin tuna collected in NE

A

NE Males (based on histological classification)	Straight Fork Length (cm)	Age (years)	GSI (based on calculated weight)	Gonad Weight (g)
Minimum	78	1.40	0.20	2.3
Maximum	130	2.71	0.85	38.1
Mean	109	2.15	0.34	9.52
Standard Deviation	18	0.46	0.17	7.60
Number of samples	19			

B

Stage		Number	Range of Straight Fork Lengths of Fish (cm)	Diameter of largest cyst or duct range (µm)
1	Primary spermatogonia	4	78-116	30-70
2	Primary spermatocytes	8	84-132	50-55
3	Secondary spermatocytes	5	107-127	45-60
4	Spermatids	0		
5	Spermatozoa	2	127-130	150

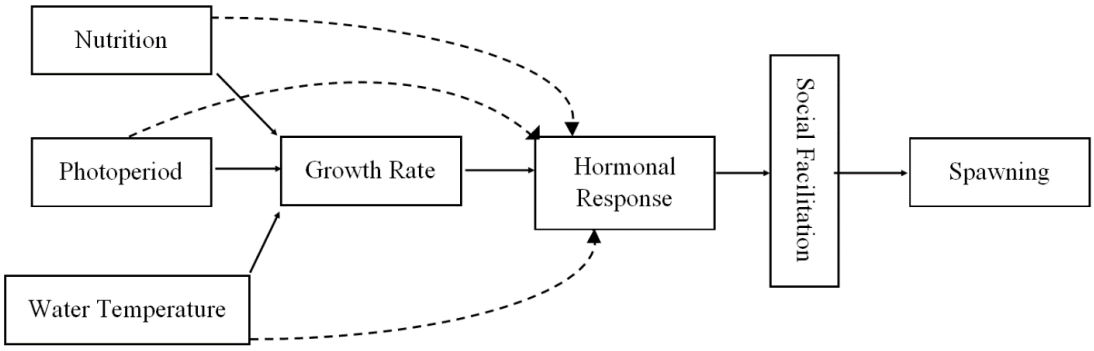
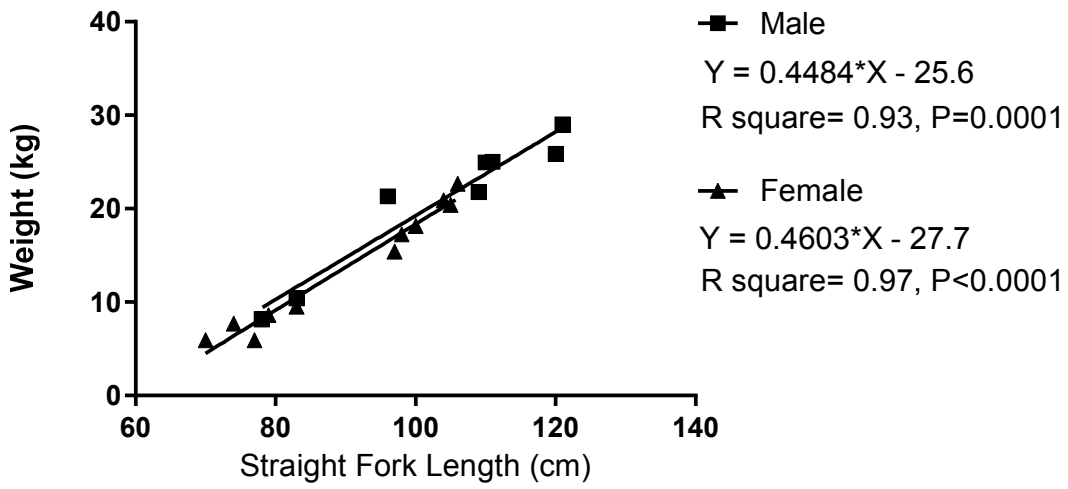


Figure 1: Path analysis of factors affecting YFT growth and development. Solid lines represent direct relations and dashed lines represent potential relations.

A



B

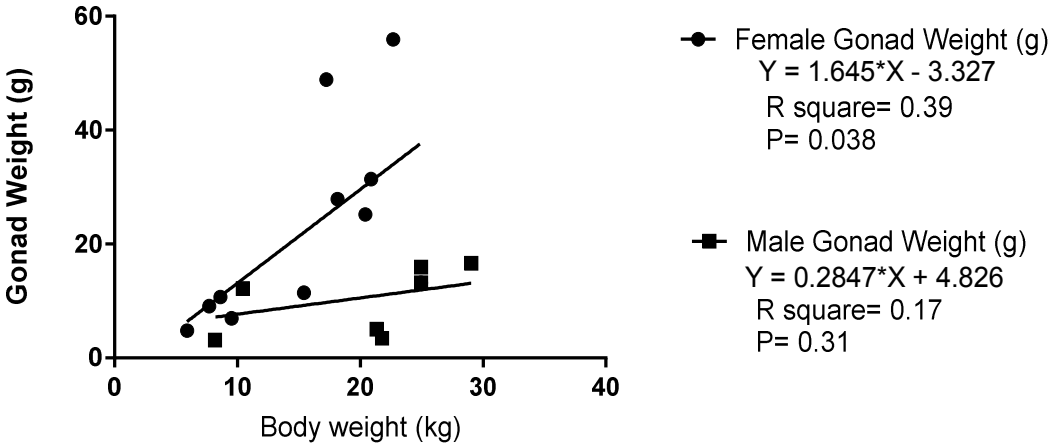


Figure 2: A) Weight of YFT in kilograms versus straight fork length in centimeters. Sex is based on histological examination of samples. All samples are from North Carolina where weight was taken using a Toledo floor scale. B) NC yellowfin tuna total body weight in kilograms vs. gonad weight in grams separated by sex.

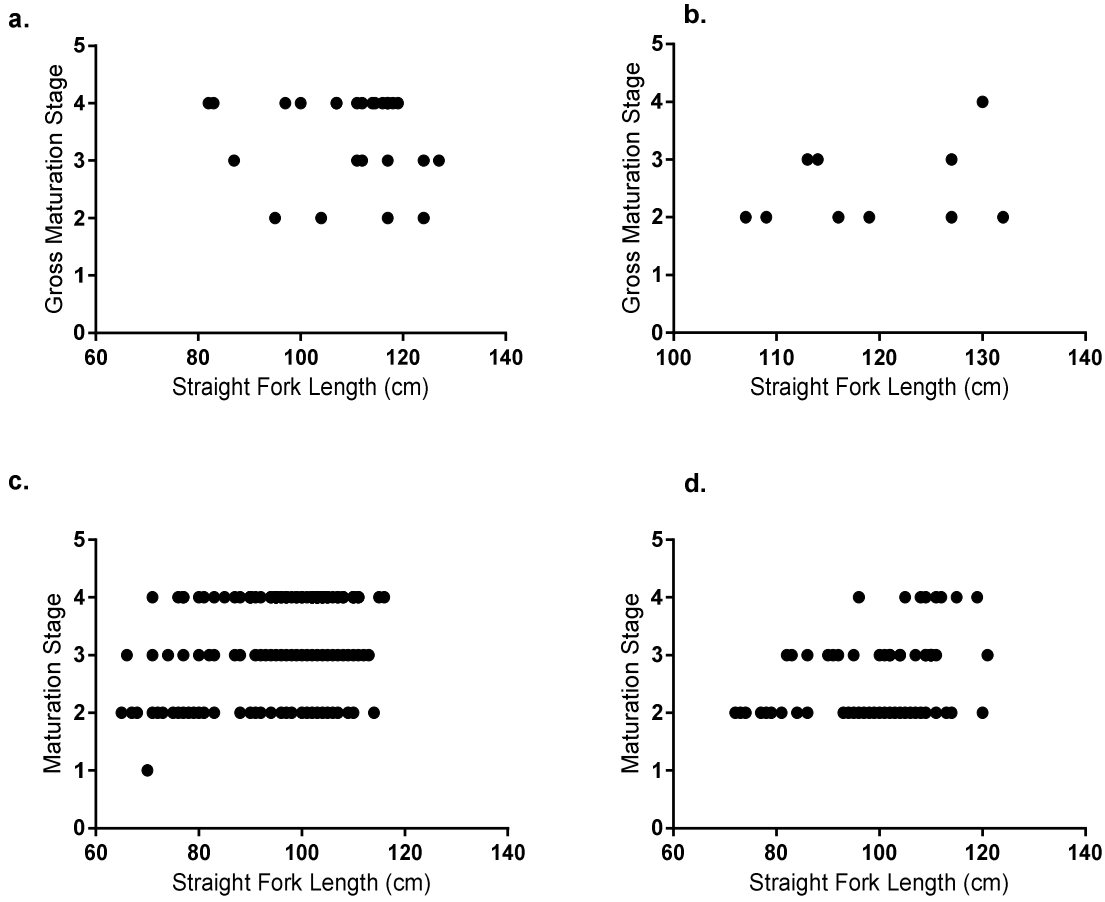


Figure 3: Straight fork length and gross maturation index (GMI) for a) NE female, b) NE male, c) NC female and d) NC male yellowfin tuna. One-way ANOVA between maturation stages was not significant for NE females ($p=0.64$) and NE males ($p=0.52$), but it was significant ($p<0.05$) for NC females ($p= 0.02$) and NC males ($p <0.001$).

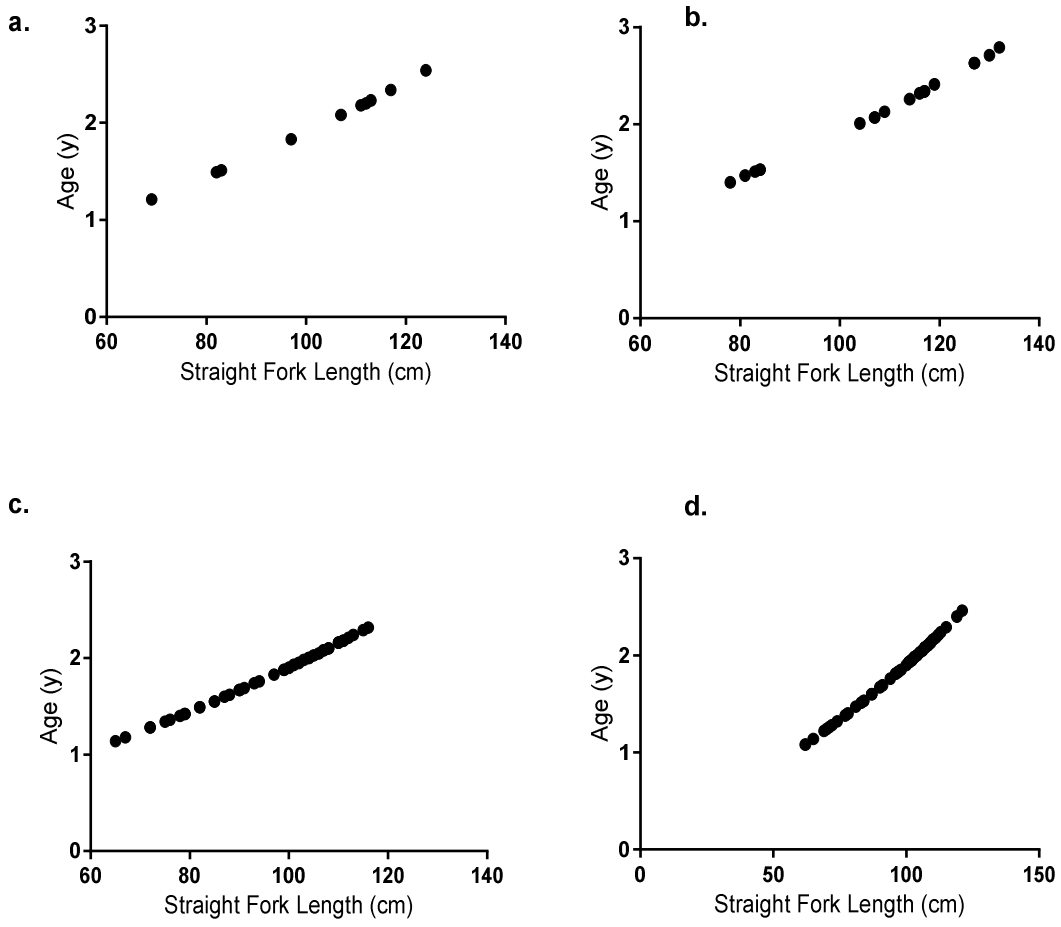


Figure 4: Straight fork length in cm versus age in years. Age is determined by $SFL = (245.541 * (1 - exp(-0.281 * (t - 0.0423))$, where t is time in years (Shuford *et al.,* 2007). Locations and sexes are a) NE female YFT, b) NE male YFT, c) NC female YFT, d) NC male YFT.

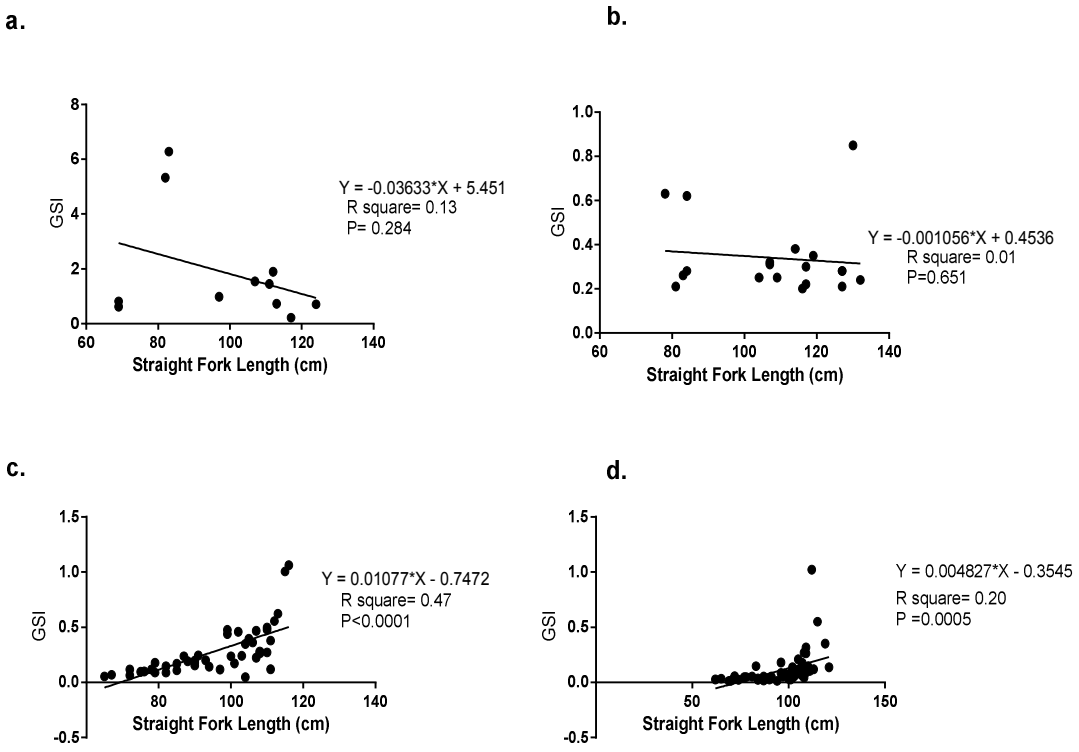


Figure 5: Straight fork length in cm versus gonadosomatic index (GSI). GSI is based on gonad weight and total weight of the fish. Weight is calculated by $W_f = a * SFL^b$ where a is1.886 x 10^{-5}, SFL is the straight fork length of the fish, and b is 3.0195 based on a sample of 6,752 YFT caught in the Indian Ocean (Marsac *et al.,* 2006) and GSI is calculated by $GSI = W_g/W_f * 100$, where W_g is the weight of gonads in grams, and W_f is the total weight of the fish including gonads (Stequert *et al.,* 2001). Locations and sexes are a) NE female YFT, b) NE male YFT, c) NC female YFT, d) NC male YFT.

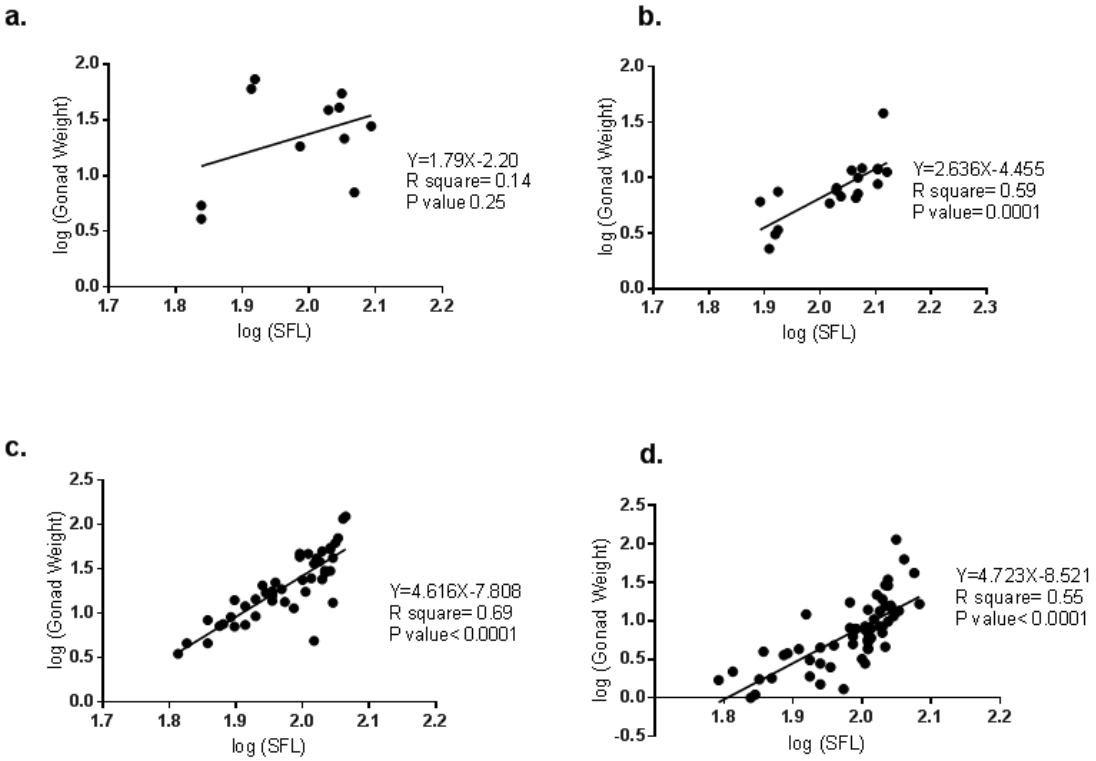


Figure 6: Log straight fork length in cm versus log of gonad weight in grams. Locations and sexes are a) NE female YFT, b) NE male YFT, c) NC female YFT, d) NC male YFT

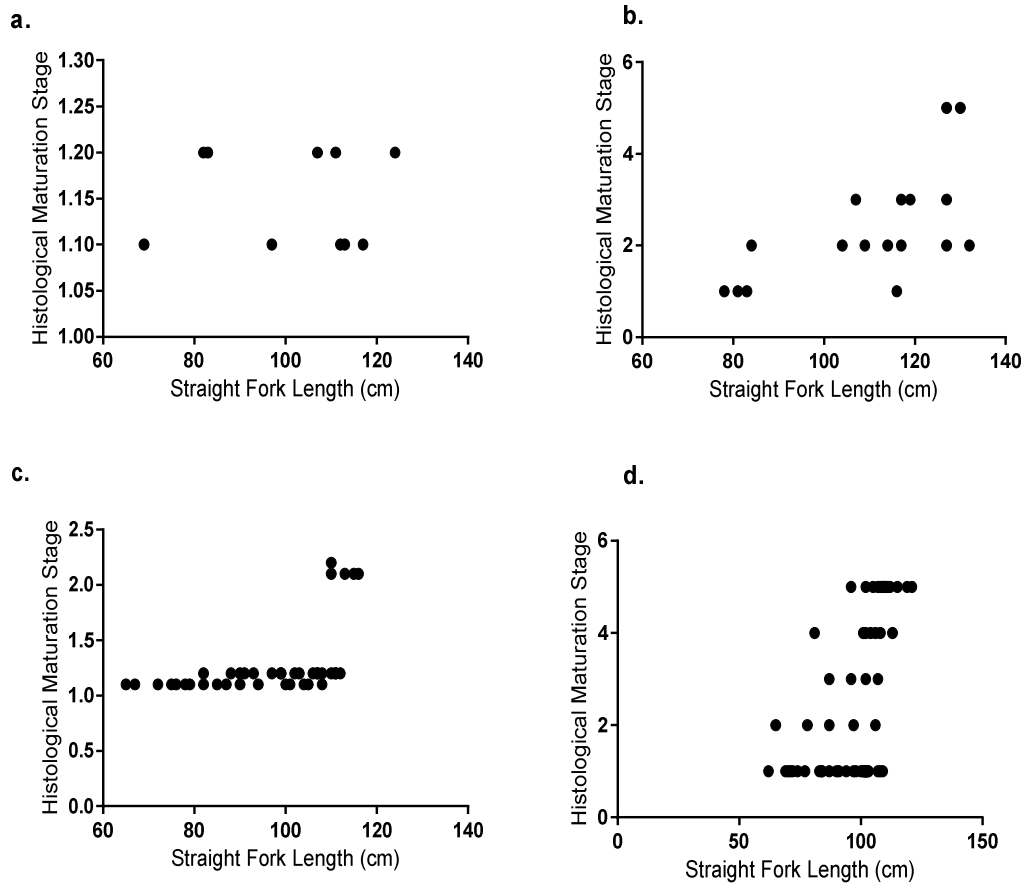


Figure 7: Straight fork length in cm versus histological maturation stage. Locations and sexes are a) New England female YFT, b) NE male YFT, c) NC female YFT, d) NC male YFT. One-way ANOVA analyses were run on each of the maturation stages in each location for each sex. Differences between mean sizes for female YFT from NE were not statistically significant, but they were statistically significant for males from NE, females from NC, and males from NC ($P < 0.05$).

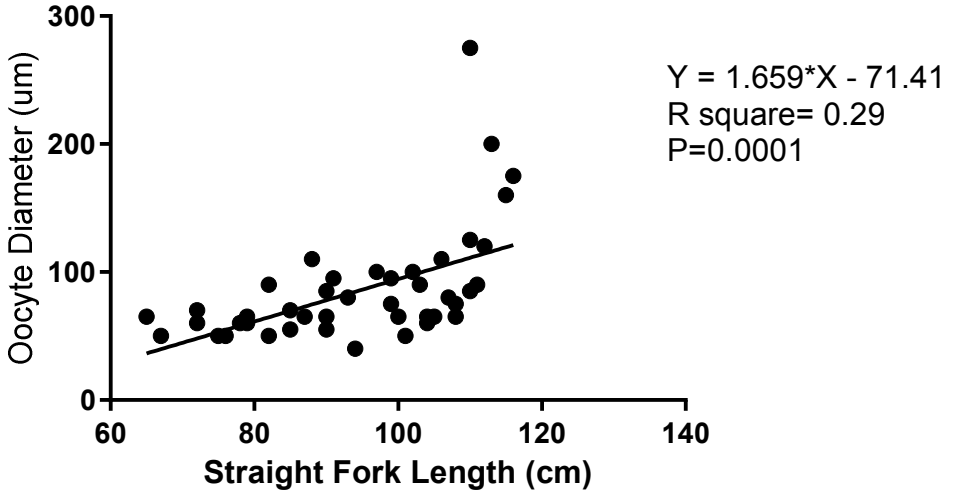


Figure 8: Straight fork length and maximum oocyte diameter in female yellowfin tuna from NC.