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On the reproductive biology of the western Atlantic bluefin tuna, Thunnus thynnus

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On the reproductive biology of the western Atlantic bluefin tuna, *Thunnus thynnus*

BY

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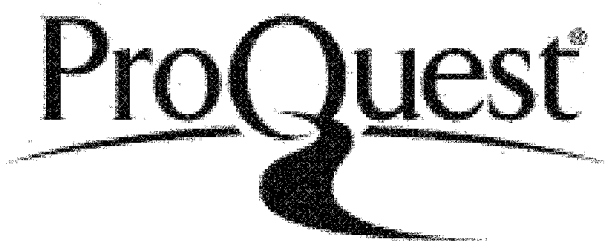

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ABSTRACT

On the reproductive biology of the western Atlantic bluefin tuna, *Thunnus thynnus*

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The Atlantic bluefin tuna, *Thunnus thynnus*, is a highly migratory species capable of traversing great distances throughout the North Atlantic Ocean, but spawning is known to occur only in the Mediterranean Sea and the Gulf of Mexico/Straits of Florida. The regulatory body charged with managing Atlantic bluefin tuna, the International Commission for the Conservation of Atlantic Tunas (ICCAT), currently recognizes two spawning stocks, eastern and western, separated by a management line at 45° W. The eastern stock spawns from May through July in the Mediterranean Sea with an age at first maturity of 3–4 years. Due in part to a moratorium on fishing for bluefin tuna in the Gulf of Mexico, considerably less research has been conducted on the western spawning stock, and, subsequently, knowledge about basic biological characteristics of this stock is lacking. The age at maturity for western bluefin tuna has been reported as 5–16 years and is a topic of debate among fisheries managers. For stock assessment and management, ICCAT assumes an age at maturity for western bluefin tuna of 9 years. While only two spawning grounds are known, fish of reproductively mature size routinely do not return to either of these two locations during the presumed spawning season indicating additional spawning grounds may exist.

Atlantic bluefin tuna were sampled on and off the known spawning grounds, and maturity status was determined for male and female fish. All fish sampled on the spawning grounds (n=250) had mature gonads, and the spawning season in the north/central Gulf of Mexico was defined as April–June. Histological analyses showed

a peak in oocyte maturation, and thus spawning activity, in May in the Gulf of Mexico sampling region.

Actively spawning fish from the Mediterranean Sea were compared with those from the Gulf of Mexico. Realized fecundity and spawning periodicity were found to be similar for both stocks, but the western spawning stock sampled in the north/central Gulf of Mexico spawns one month earlier than the eastern stock.

Fish sampled far from the known spawning grounds provided further information about the reproductive condition of western bluefin tuna. The youngest female and male to show signs of maturity had estimated ages of 6 years and 5 years, respectively. About one quarter of all females sampled contained vitellogenic or early atretic oocytes, and based on rates of atresia, it is unlikely these fish spawned in the Gulf of Mexico.

These results provide more extensive information about the reproductive biology of western Atlantic bluefin tuna and revise the age at maturity for the western spawning stock. This lower size and age at maturity, coupled with new results from endocrinology and electronic tagging data, suggests alternative spawning grounds exist, and more comprehensive spatial and temporal sampling is necessary to understand the reproductive biology of Atlantic bluefin tuna.

CHAPTER 1

INTRODUCTION

Considering the amount of research which has been devoted to the Atlantic bluefin tuna, positive information on its spawning habits is surprising incomplete.

Almost 20 years later, the above quote from Mather et al. (1995) still holds true. The Atlantic bluefin tuna, *Thunnus thynnus* Linneaus, 1758, is a depleted stock and a highly valuable commercial and recreational fisheries resource (Mather et al., 1995; Sissenwine et al., 1998; Rooker et al., 2007) that has been fished since ancient times (Sara, 1980). With the worldwide increase in demand, fishing pressures are at an all time high, and bluefin tuna are among the most valuable fish in the ocean (Bestor, 2004). Historically, bluefin tuna occurred from Newfoundland to Brazil in the western Atlantic and from Norway to the northern coast of Africa in the eastern Atlantic (Hamre, 1958, 1960; Hamre et al., 1968; Tiews, 1978; Mather et al., 1995). Tuna distribution is not random (Nakamura, 1965, 1969; Sund et al., 1981; Sibert & Fournier, 1994; Sibert et al., 1996, 1999; Sharp, 2001) and fish usually associate with specific oceanographic features and environmental characteristics suitable for reproductive and foraging success (Cushing, 1982; Sinclair & Iles, 1985; MacCall, 1990; Mann, 1993). Advances in fishing gear technology have exploited this non-random distribution and have allowed year-round exploitation of the remaining stock throughout most of its range, including some of the spawning grounds. Because of the highly migratory nature of bluefin tuna, US state and federal management alone proved inadequate (Mather et al., 1995). Following political pressure, international

management began in the 1970's with the formation of the International Commission for the Conservation of Atlantic Tunas (ICCAT).

Initially, ICCAT managed Atlantic bluefin tuna as one stock; however, in 1976, increasing political pressure resulted in the Commission considering the two stock hypothesis (Whynott, 1995; Fromentin & Powers, 2005). This hypothesis was adopted in 1980, a management boundary at 45° W was established, and ICCAT began assessing separate spawning stock biomasses (SSB) for the newly established 'eastern' and 'western' stock units (Parrack, 1980, 1982). The basis for management was the knowledge of two separate and exclusive spawning locations and low levels of mixing (Sella, 1930; Rivas, 1954; Tiews, 1963; Sara, 1964; Mather et al., 1974; Richards, 1976; Montolio & Juarez, 1977). Research after 1980 supported this split (Piccinetti & Piccinetti-Manfrin, 1993; Mather et al., 1995), but more recent electronic tagging results suggest the two-stock hypothesis may not be appropriate (Fromentin & Powers, 2005; Fromentin, 2009; Galuardi et al., 2010), and a new management scheme may be required. While the Atlantic bluefin tuna stock is undoubtedly depleted (Fromentin & Powers, 2005; Safina & Klinger, 2008; ICCAT, 2009), there are still large uncertainties surrounding the stock size and structure as mixing rates between the two stocks are still unresolved (Carlsson et al., 2004, 2007; Rooker et al., 2008a). Additionally, the reproductive biology of Atlantic bluefin tuna remains poorly understood and riddled with inconsistencies in age at maturity despite the high economic value of the fishery (Goldstein et al., 2007; Lutcavage et al., 2012). A thorough understanding of the reproductive characteristics (size/age at maturity, spawning frequency, spawning periodicity, fecundity, etc.) is crucial for determining the regenerative ability of the stock (Mather et al., 1995; Quinn & Deriso, 1999; Fromentin & Powers, 2005).

Atlantic bluefin tuna are an iteroparous species having multiple spawning events in a lifetime. While it is assumed bluefin tuna spawn annually, satellite tagging data

show fish of assumed mature size outside the known spawning grounds during the presumed spawning seasons indicating bluefin tuna may not spawn annually and/or additional spawning grounds may exist (Lutcavage et al., 1999; Fromentin & Powers, 2005; Fromentin & Ravier, 2005; Rideout et al., 2005; Goldstein et al., 2007; Secor, 2007, 2008; Galuardi et al., 2010; Lutcavage et al., 2012).

The current assumption is that the Mediterranean Sea, the Gulf of Mexico, and the Straits of Florida are the sole spawning locations for bluefin tuna. Extensive research has been carried out regarding the reproductive biology of the eastern (Mediterranean Sea) spawning stock (Dicenta, 1977; Susca et al., 2001a; De Metrio et al., 2002; Medina et al., 2002; Mourente et al., 2002; Corriero et al., 2003; Santamaria et al., 2003; Viñas et al., 2003; Karakulak et al., 2004b; Corriero et al., 2005; Oray & Karakulak, 2005; Medina et al., 2007; Heinisch et al., 2008; Aranda et al., 2011, 2012). However, far less research has been conducted on the western (Gulf of Mexico) spawning stock (Rivas, 1954; Rodríguez-Roda, 1964, 1967; Richards, 1976; Montolio & Juarez, 1977; Baglin, 1982). Atlantic bluefin tuna in the eastern stock reach maturity at age 3–4 years (Corriero et al., 2005) while ICCAT assumes 100% maturity of the western stock at age 9 years (ICCAT, 2009).

However, historic studies of gonad tissues indicate western females could reach maturity at 4–6 years (Westman & Neville, 1942; Baglin, 1982). The most recent proposed increase in western bluefin tuna age at maturity to 12–16 years was based on the assumption of maturity from length data and did not examine any reproductive tissues (Diaz & Turner, 2007; Diaz, 2011). Contemporary studies on the western age at maturity utilized fish caught outside the spawning grounds (Goldstein et al., 2007) or made inferences from electronic tagging data (Block et al., 2005; Teo et al., 2007a) or catch records (Diaz & Turner, 2007; Diaz, 2011). While previous indirect techniques provide useful information, direct histological observations of gonad tissues must be

used to determine reproductive parameters such as maturity, spawning periodicity, and fecundity (Mather et al., 1995).

Understanding the reproductive biology of commercially valuable fishes is necessary for fisheries managers to accurately assess the state of the fishery and to determine the viability of rebuilding already depleted stocks. Current management schemes rely on knowing the spawning stock biomass as estimated from an accurate maturity ogive of the total population. The maturity ogive for western Atlantic bluefin tuna has not been resolved, and thus, more information is required for successful stock recovery plans.

My overall research objective was to improve the understanding of the reproductive biology of the western Atlantic bluefin tuna by sampling a wide size range of individuals over a broad spatial and temporal scale. By providing a more comprehensive biological profile of the western spawning stock, and by comparing these findings with the eastern stock, my results will increase the understanding of Atlantic bluefin tuna reproductive biology and provide a more accurate picture of the spawning population.

CHAPTER 2

REPRODUCTIVE CONDITION OF ATLANTIC BLUEFIN TUNA SAMPLED FROM KNOWN AND POTENTIAL SPAWNING AREAS

2.1 Atlantic bluefin tuna sampled from the Gulf of Mexico

Introduction

The Atlantic bluefin tuna (*Thunnus thynnus*) is a highly migratory species capable of traversing great distances throughout the North Atlantic Ocean but spawning is known to occur in only two locations: the Mediterranean Sea and the Gulf of Mexico. The regulatory body charged with managing bluefin tuna, the International Commission for the Conservation of Atlantic Tunas (ICCAT), currently recognizes two spawning stocks separated by a management line at 45° W. This separation was put in place under the assumption that there was very little mixing between the two stocks and that both stocks exhibited natal homing. However, research has shown that the two stocks mix considerably on the foraging grounds and display natal homing for spawning (Boustany et al., 2008; Rooker et al., 2008a).

Given the widespread fisheries in the Mediterranean Sea, extensive research has been carried out regarding the reproductive biology of this spawning stock including the parameters that define reproductive potential such as fecundity, spawning frequency, and sex-ratio (Susca et al., 2001b; De Metrio et al., 2002; Mourente et al., 2002; Medina et al., 2002, 2007; Santamaria et al., 2003; Viñas et al., 2003; Corriero et al., 2003, 2005; Abascal et al., 2004; Karakulak et al., 2004a,b; Oray & Karakulak, 2005; Aranda et al., 2011, 2012). The eastern stock spawns from May through

July in the Mediterranean Sea with age at first maturity being established at 3–4 years (Cort, 1991; Corriero et al., 2005). Four specific spawning areas within the Mediterranean Sea include the Balearic Sea, Malta Island, the South Tyrrenhian Sea, and the Levantine Sea (Nishida et al., 1998; Susca et al., 2001a; Medina et al., 2002; Corriero et al., 2003; Karakulak et al., 2004b). In the western and central Mediterranean Sea (Balearic Islands, Malta Island, and South Tyrrenhian Sea), bluefin tuna reach peak spawning conditions in June and July while in the eastern Mediterranean Sea (Levantine Sea), bluefin tuna spawn in May and June (Karakulak et al., 2004b; Heinisch et al., 2008). Such detailed knowledge of the temporal and spatial variability in bluefin tuna spawning in the Mediterranean Sea provides valuable insight into the reproductive dynamics of this stock, but similar data are lacking for the western spawning stock.

Due, in part, to a moratorium on directed fishing for bluefin tuna in the Gulf of Mexico, considerably less research has been conducted on the western spawning stock, and, subsequently, our knowledge about basic biological principles of this stock is lacking. Larval surveys (Richards, 1976; Montolio & Juarez, 1977) and macroscopic ovary examinations (Rivas, 1954; Baglin, 1982) have identified the central Gulf of Mexico and the Florida Straits as the localized spawning areas for the western stock but a comprehensive spatial sampling for histological examination of spawning bluefin tuna has not been conducted. The age at maturity for western bluefin tuna has been reported as 5–16 years and is a topic of intense debate among fisheries managers. Historical age at maturity studies indicate females from the western spawning stock could mature at age 5–6 years (Westman & Neville, 1942; Baglin, 1982). Goldstein et al. (2007) showed evidence of maturity (but not actual spawning) in fish 7–8 years old; however this study was conducted on the New England foraging grounds far from the known spawning grounds. Recent studies proposing an increase in the age

at maturity to 12–16 years (Diaz & Turner, 2007; Diaz, 2011) did not examine any gonad tissues to confirm maturity but assumed maturity based on ages estimated from lengths of bluefin tuna collected as bycatch from the longline fisheries operating in the north/central region of the Gulf of Mexico in the spring. Despite these discrepancies, ICCAT assumes an age at 100% maturity for the western spawning stock of 9 years (Anonymous, 2011) and does not account for any temporal or spatial variability in spawning within the Gulf of Mexico.

While the spatially and temporally limited historic sampling provides some information about the spawning activities in the Gulf of Mexico and Straits of Florida, there are still many reproduction related uncertainties for the western stock. Based on histological analyses of gonads, the spawning season in the Gulf of Mexico is believed to peak in May (Rivas, 1954; Baglin, 1982). Fish smaller/younger than the presumed size/age at maturity (176 cm, 9 yrs) have not been sampled from the Gulf of Mexico leading to suggestions that additional spawning grounds may exist (e.g., Bahamas, Caribbean Sea, Mid-Atlantic Bight) for smaller bluefin tuna (Mather et al., 1995; Lutcavage et al., 1999). Galuardi et al. (2010) showed fish entering the Gulf of Mexico as early as November indicating fish may be foraging in the Gulf of Mexico (de Buen, 1925) or may be spawning earlier than previously documented. Bluefin tuna larval development requires a minimum temperature of 21°C with a preference for temperatures closer to 24°C (Rivas, 1955; Piccinetti & Piccinetti-Manfrin, 1993; Nishida et al., 1998; García et al., 2003, 2005, 2007; Alemany et al., 2010). The lowest temperature at which bluefin tuna initiate spawning is 20.5°C (Alemany et al., 2010), and additional tagging studies show many fish of assumed reproductively mature size outside either of the two known spawning areas, but in areas with sea surface temperatures above 24°C, during the known spawning season (Lutcavage et al., 1999; Block

et al., 2005; Galuardi et al., 2010). The lack of spatially and temporally consistent spawning patterns in adult bluefin tuna indicate two possible hypotheses:

1. Bluefin tuna may not spawn annually. Skipped spawning has been identified in a number of fish species (Rideout et al., 2005; Jørgensen et al., 2006; Rideout & Tomkiewicz, 2011), and while egg production is energetically costly, bluefin tuna on the spawning grounds with massive atresia or those not returning to the spawning grounds may be reserving energy for future spawning seasons (Rideout et al., 2000, 2005; Rideout & Tomkiewicz, 2011).

2. Bluefin tuna may be utilizing alternative spawning grounds. The lack of smaller fish represented in the Gulf of Mexico longline catches suggests fish may be segregating by size both within and outside the known Gulf of Mexico spawning grounds. Such size segregation has been observed for bluefin tuna in the Mediterranean Sea (Heinisch et al., 2008) and for Pacific bluefin tuna, *Thunnus orientalis* (Itoh, 2006).

Making inferences from depth patterns from electronic tagging data, Teo et al. (2007a) interpreted changes in diving behavior as an indication of spawning in the Gulf of Mexico. Macroscopic examination of gonads has also been used to determine maturity and/or spawning (Westman & Neville, 1942). While these methods provided information about the migratory patterns of bluefin tuna on the spawning grounds, they are not sufficient for accurately defining the reproductive condition of fish or the temporal dynamics of the spawning season. Direct histological examination of gonad tissue is required for observing the different stages of follicle development that define maturation and, thus, the bounds of the spawning season (Medina et al., 2002). Using direct gonad examination, 96% of fish sampled in the Bahamas (Rivas, 1954; Baglin, 1976) and the Mid-Atlantic Bight (Baglin, 1982) were in post-spawning condition, but no ripe females were sampled from the Mid-Atlantic Bight. Since the samples

were taken far from the known spawning grounds, these studies were limited and could not provide information about the reproductive condition of actively spawning fish, such as spawning frequency and/or fecundity (Clay, 1991).

Fecundity estimates allow the quantification of the reproductive capacity of individual fish and are essential for accurate assessment of the spawning stock (Murua et al., 2003). Assuming environmental characteristics between the Mediterranean Sea and the Gulf of Mexico spawning grounds are different, and that bluefin tuna exhibit natal homing, fecundity must be calculated separately for each stock. Realized and potential batch fecundities can be calculated by stereological counts of post-ovulatory follicles (POFs) and migratory-nucleus follicles (MNF), respectively (Aragón et al., 2010; Aranda et al., 2011). As both these stages of follicular development are present in bluefin tuna ovaries for less than 24 hours, it is essential that sampling be conducted on the spawning grounds.

The main objectives of this study are to assess the maturity status of male and female Atlantic bluefin tuna sampled from the western spawning grounds to define reproductive traits such as maturity stage, fecundity, spawning periodicity, and spawning frequency. None of these parameters have been directly assessed for the western spawning stock in the past three decades, and more recent studies have relied on indirect assessment (electronic tag data, larval surveys, etc.). This study builds on earlier works and integrates related studies on fish condition, migration, etc. to more fully evaluate the reproductive biology of this economically valuable and highly depleted species.

Methods

T. thynnus were sampled from the Gulf of Mexico spawning grounds. The Gulf of Mexico is located in the southeastern corner of North America and is bordered by the

US to the north (Florida, Alabama, Mississippi, Louisiana, and Texas), Mexico to the west (Tamaulipas, Veracruz, Tabasco, Campeche, and Yucatan), and the island of Cuba to the south. The Gulf of Mexico measures about 1600 km from east to west, and 900 km from north to south. The continental shelf and slope have water depths of < 180 m and $180 - 3,000$ m, respectively (Figure 2-4).

Sample Collection

Samples of male and female bluefin tuna gonads were collected between 2007 and 2009 from the Gulf of Mexico spawning grounds. Fish were sampled on board as bycatch from the yellowfin tuna and swordfish (*T. albacares* and *Xiphias gladius*, respectively) fisheries by National Marine Fisheries Service (NMFS) observers based mostly out of Houma, LA. Curved fork length (CFL) was measured to the nearest centimeter, though discrepancies in the length measurement methods existed and were not always documented in the sampling notes (i.e., straight vs. curved fork length, fin length, etc.). For body weight (BW) and age estimations, CFL was converted to straight fork length (SFL; Eqn. 2.1; Parrack & Phares, 1979). Upon landing, dressed weight (DW; body weight minus head, tail, and internal organs) was measured to the nearest kilogram and was converted to total body weight (BW; Eqn. 2.2). When DW was not measured, BW was calculated from SFL based on time of catch according to ICCAT conversion factors (Table. 2.1). All weights and lengths are reported as BW and CFL, respectively, unless otherwise stated. The Gonadosomatic Index (GSI; Eqn 2.3) was calculated using the gonad weight (GW) and BW for each fish.

$$SFL = CFL \cdot 0.955 \tag{2.1}$$

$$BW = DW \cdot 1.35 \tag{2.2}$$

$$GSI = 100 \cdot \frac{GW}{BW} \tag{2.3}$$

Month	Body weight conversion
Feb–March	$BW = 2.861 \cdot 10^{-5} SFL^{2.929}$
April–May	$BW = 6.043 \cdot 10^{-5} SFL^{2.7794}$
June	$BW = 4.404 \cdot 10^{-5} SFL^{2.837}$

Table 2.1. Monthly ICCAT conversion equations used to estimate body weight (BW) from dressed weight (DW) of Atlantic bluefin tuna, *Thunnus thynnus*, sampled in the Gulf of Mexico.

Histology and Stereology

Whole gonads were removed from the body cavity upon capture and weighed to the nearest kilogram. Sex was determined by macroscopic examination of the gonads (Figure 2-1). Subsamples were excised from the middle of the gonad and fixed in 10% neutral buffered formalin. Tissue samples were rinsed and stored in 70% ethyl alcohol (EtOH), dehydrated in a series of increasing concentrations of EtOH, and cleared with ClearRite3®. Tissue samples were embedded in paraffin wax, sectioned to 5 μm sections, stained with haematoxylin and eosin, mounted on glass slides using a high clarity mounting medium. Maturity status for both males and females was determined by examining the entire slide using a compound microscope (40–100x).

Stereology allows the estimation of the proportion, and thereby the abundance, of different oocyte stages within the ovary. The model-based stereology techniques applied in this study are a modified version of those described by Coward & Bromage (2002) according to Weibel & Gomez (1962) (Eqn. 2.4) and previously used on bluefin tuna by Medina et al. (2002, 2007) and Aragón et al. (2010). For this study, stereology was used to determine both spawning frequency and fecundity. A more comprehensive discussion of stereology (including the details of the equation below) will be presented in Chapter 3.

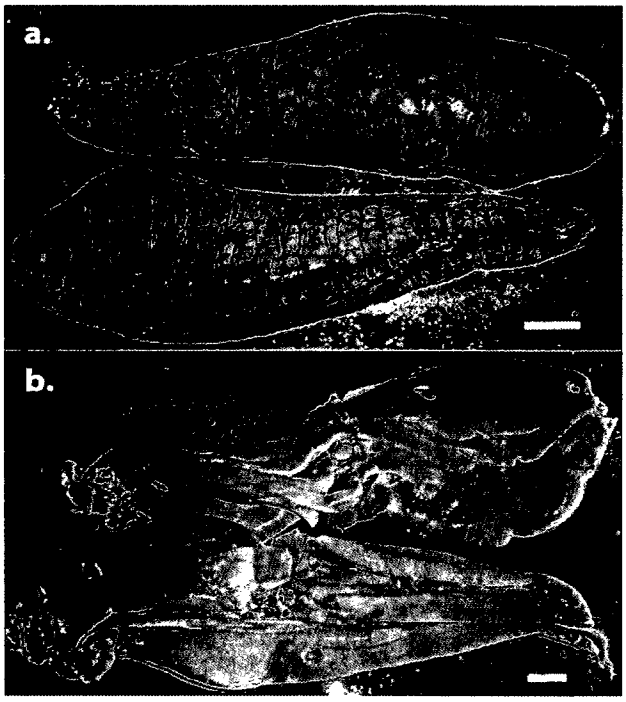

Figure 2-1. Whole gonads from Atlantic bluefin tuna, *Thunnus thynnus*, from the Gulf of Mexico: (a) female, 4.5 kg ovaries sampled from a 180 cm, 99 kg fish; (b) male, 11.2 kg testes sampled from a 271 cm, 377 kg fish. Scale bar is 5 cm.

$$N_V = \frac{K N_A^{1.5}}{\beta V_V^{0.5}} \tag{2.4}$$

Female classification

Maturity status for females was assessed by determining the most advanced oocyte stage present in each sample (Schaefer, 1998; Table 2.2). In bluefin tuna, ovarian development is considered asynchronous since oocytes in various stages of development are simultaneously present in the ovary. Histological analysis of ovaries was used to classify females as active or inactive. Females were classified as active if the ovary contained advanced yolked oocytes, and there was little to no α atresia present. The onset of α-atresia is characterized by the disintegration of the nucleus and some yolk globules combined with the disintegration of the zona radiata. These characteristics are identified using histology by irregular shape and changes in staining efficacy (Hunter & Macewicz, 1985). Active females were then further classified as non-spawning or spawning. Ovaries showing evidence of imminent spawning (migratory-nucleus and/or hydrated oocytes) or recent spawning (post-ovulatory follicles) were classified as spawning, and females classified as active without either of these criteria were classified as non-spawning. All females classified as active are reproductively mature.

Females classified as inactive were further classified as either immature or mature as an active mature female can reabsorb maturing oocytes and regress to the inactive state. Females with unyolked or early yolked oocytes combined with α and/or β atresia were classified as inactive-mature since these females showed signs of previous reproductive activity (follicle maturation). Females were also classified as inactive-mature if the ovaries contained advanced yolked oocytes *and* contained more than 50% atretic follicles (major atresia). Fish were classified as inactive-immature if they contained unyolked or early yolked oocytes but lacked any signs of atresia. Females

classified as inactive could be reproductively immature or reproductively mature but in a regressed stage.

Spawning frequency was estimated from active-spawning fish using the post-ovulatory follicle method (Eqn. 2.5) as this method corrects for biases in the numbers of females with hydrated oocytes (Stauffer & Picquelle, 1981; Hunter & Macewicz, 1985),

$$F = \frac{M_{li}}{2M_{li} + m_{ni}} \tag{2.5}$$

where, F = fraction of females spawning per day, M_{li} = number of females with 1-day old post-ovulatory follicles (POFs), and m_{ni} = the number of females with no spawning history (females with POFs and/or hydrated or migratory nucleus oocytes are excluded). For bluefin tuna, POFs are assumed to reabsorb within 24h as is seen with yellowfin and skipjack tuna (Hunter et al., 1986; McPherson, 1991; Schaefer, 1996) so F is referred to as the proportion of females spawning within a 24h period.

Fecundity was determined by the number of migratory-nucleus and/or hydrated oocytes (final maturation oocytes) in addition to the number of POFs present in the ovary. For each female, stereology was used to determine the number of final maturation oocytes and POFs for a given volume. The entire gonad volume (GV) was calculated from the gonad weight (GW; Eqn. 2.6; Medina et al., 2007), and the fecundity was extrapolated from the stereological measurements to include the whole gonad volume. Gonad volume loss has been documented as a result of processing (Medina et al., 2007; Aranda et al., 2011). For our samples, we calculated volume loss through processing and applied a correction factor of 43.4%.

$$GV = GW \cdot 0.9174 \tag{2.6}$$

Ovarian classification		Histological features
Active	Nonspawning	Advanced yolked oocytes and minor, if any, α atresia
	Spawning	Advanced yolked oocytes, minor α atresia, plus postovulatory follicles and/or migratory nucleus oocytes and/or hydrated oocytes
Inactive	Immature	Previtellogenic or early yolked oocytes plus no atresia
	Mature	Previtellogenic or early yolked oocytes plus α and β atresia, or advanced yolked oocytes plus major atresia

Table 2.2. Maturity classifications assigned to female Atlantic bluefin tuna, *Thunnus thynnus*, based on ovarian histology according to Schaefer (1998).

Male classification

Bluefin tuna testis are classified as unrestricted spermatogonial type since the distribution of spermatogonia occurs along the entire length of the tubule (Grier, 1981; Abascal et al., 2004). Histological evidence of recent spawning in male bluefin tuna is only visible for about 12h after spawning (Schaefer, 1998). The stages differentiated and recorded in maturity determination were, in increasing order of maturity: spermatogonia, spermatocytes, spermatids, and spermatozoa. Males were classified as immature if there were no spermatozoa in the sperm duct. Mature males were classified according to Santamaria et al. (2003) and Abascal et al. (2004) by determining the most advanced stage of spermatogenesis present as well as identifying the presence or absence of spermatozoa (milt) in the central duct (Table 2.3).

Age determination

We estimated age separately from both SFL and BW according to Restrepo et al. (2010). We then measured age directly by counting annual growth rings on the first spiniform ray of the first dorsal spine (herein: dorsal spine).

Testis classification	Histological features
Quiescence	Spermatogonia and spermatocysts with few, if any, spermatids.
Early spermatogenesis	Germ cells at all stages of spermatogenesis with more spermatocytes and spermatids; only a few spermatozoa
Late spermatogenesis	Many spermatids; more abundant spermatozoa in ducts than in previous stages
Spawning	Lumen all filled with spermatozoa; residual spermatids present in the periphery
Regression	Lumen almost devoid of spermatozoa with some residual spermatozoa in the ducts

Table 2.3. Maturity classifications assigned to male Atlantic bluefin tuna, *Thunnus thynnus*, based on testis histology according to Santamaria et al. (2003).

The intact first dorsal spine, including the condyle, was removed from each fish upon landing. After removing the skin and any connective tissues, the dorsal spine was stored until dried. The location for sectioning the dorsal spine was standardized with previous studies by measuring the anterior diameter of the dorsal spine along an axis just above the hollows then measuring dorsally one and one half times the previously measured diameter (Figure 2-2). The spine was cut into 0.7 mm sections using a low speed Isomet saw with a diamond wayfaring blade. Sections were then washed in 70% EtOH and mounted to glass slides using Eukitt Mounting Medium, a highly transparent resin (Electron Microscopy Sciences, Hatfield, PA). Spine sections were viewed under transmitted light using a binocular lens dissecting microscope with a digital camera mounted on the top of the scope. Images were taken of each spine and analyzed using ImageJ software (Rasband, 1997–2011).

The growth bands are clearly visible on bluefin tuna dorsal spine sections and can be separated as narrow translucent bands indicating slow winter growth or wide opaque zones indicating fast summer and fall growth associated with feeding (Compean-Jimenez & Bard, 1980; Cort, 1991). Using marginal increment analysis, Cort (1991)

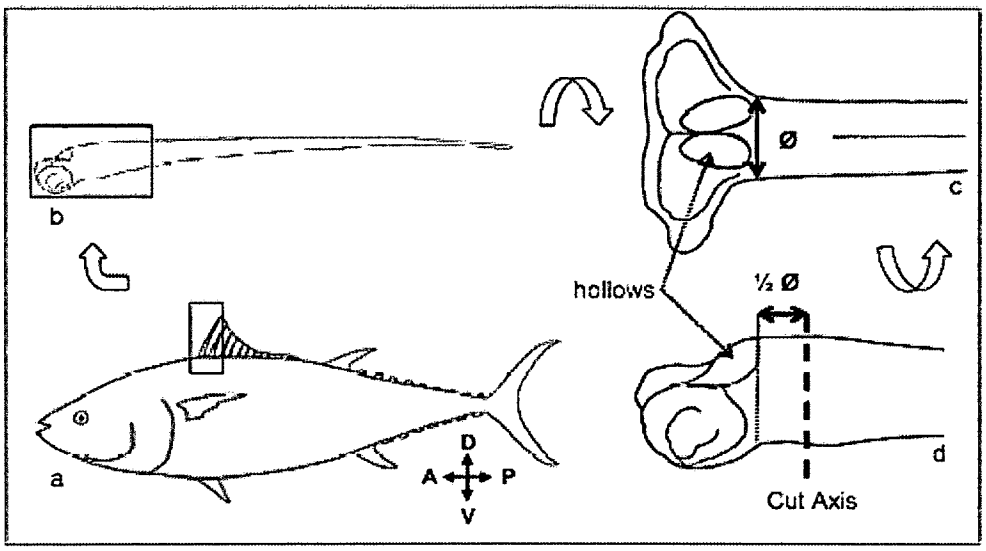

Figure 2-2. A graphical depiction of the standardization of the cutting axis location reproduced from Rodríguez-Marín et al. (2007). (a) lateral view of the entire bluefin tuna with the location of the first dorsal fin ray indicated by the rectangle. (b) lateral view of the first dorsal fin ray after removal from the fish. (c) dorsal view of the base of the fin ray with θ indicating the location of the diameter measurement just behind the hollows. (d) lateral view of the fin ray with the location of the cut access marked at 0.5θ. For this study, the cut was made at 1.5θ as this distance has been shown to give greater clarity in ring visibility (Rodríguez Marín, pers. comm.). A=anterior, P=posterior, D=dorsal, V=ventral.

showed that translucent bands are accumulated annually and are indicators of annual growth. As fish age (greater than 3 years old), it becomes more difficult to accurately determine age as the central nucleus of the spine, including the first annulus, is re-absorbed (Compean-Jimenez & Bard, 1980; Cort, 1991; Megalofonou & De Metrio, 2000). To account for this, the diameter of the first visible translucent ring was measured and compared with mean values of previously measured rings on younger fish (Cort, 1991). This technique is dependent on a strong correlation between fish length and maximum spine diameter (Compean-Jimenez & Bard, 1980; Corriero et al., 2005; Cort, 1991; Rodríguez-Marín et al., 2006). Once the age of the first translucent ring

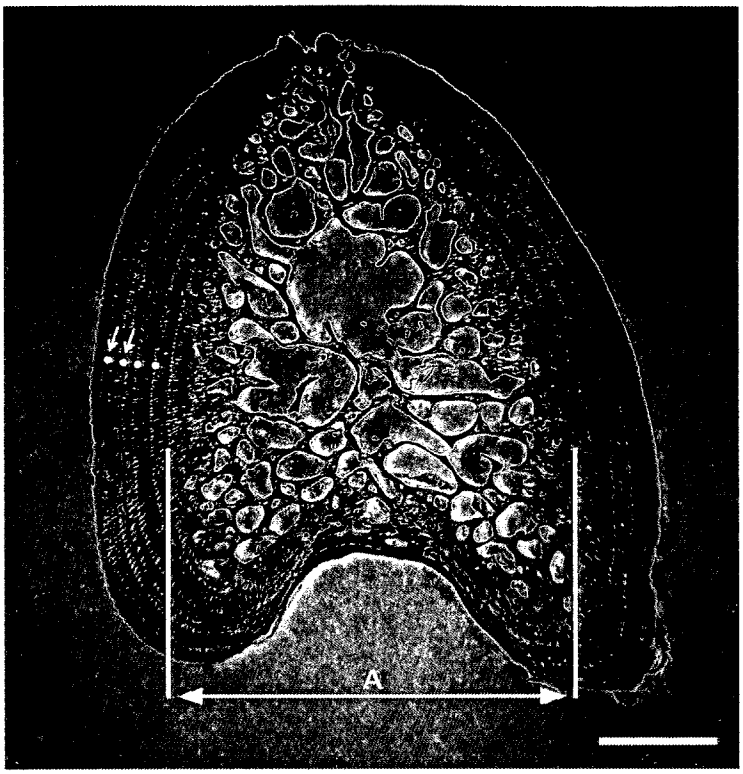

Figure 2-3. A cross section of a dorsal spine from a 12 year old fish. (A) marks the diameter of the first visible translucent ring (9.518 mm), and further rings are marked with dots. Doublets are indicated by arrows. Scale bar is 3 mm.

is determined, subsequent rings are counted annually to calculate the age of each fish (Figure 2-3).

Statistics

All statistical analyses were conducted using JMP statistical software (SAS Institute Inc., 1989-2010). Equal variance was confirmed with the O'Brien test. If variances were equal, Tukey-Kramer HSD was used to compare all means with Bonferroni corrections for multiple comparisons ($\alpha = 0.0125$). If variances were unequal, the Kruskal-Wallis test was used for a nonparametric comparison ($\alpha = 0.05$). Student's

t-test was used to compare two means ($\alpha = 0.05$; Sokal & Rohlf, 1998; Zar, 1999).

Results

From 2007 to 2009, 250 gonad samples were collected by NMFS observers on longline fishing vessels operating in the northern part of the Gulf of Mexico (Figure 2-4). With the exception of four fish, all fish sampled on the spawning grounds were 'giants' (i.e., $>$ 205 cm, curved fork length (CFL); Figure 2-5). Total body weight (BW) ranged from 99 to 582 kg (Table 2.4). There was no statistical difference between years for BW or CFL (Tukey-Kramer HSD, $\alpha = 0.05$). There was a significant difference for BW between April and May with fish in May being smaller than those in April (Tukey-Kramer HSD, $\alpha = 0.05$). There was also a significant difference for CFL between May and June (Tukey-Kramer HSD, $\alpha = 0.05$) with fish caught in June being longer than those caught in May. When comparing males to females, there was no significant difference in CFL, but males were, on average, heavier than females (Tukey-Kramer HSD, $\alpha = 0.05$).

The sex ratio of fish caught was skewed with 62% female and 38% male. Gonad size varied from 0.9–16.9 kg (Table 2.4) with no significant difference in either male or female gonad weight between months or years (Tukey-Kramer HSD, $\alpha = 0.05$). GSI values ranged from 0.35–7.25 with small peaks for females in early and mid May (Figure 2-6). Male GSI increasd throughout the sampling period then began to fall toward the end of the spawning season. Male and female fish had significantly different GSI values (student's t test, $\alpha = 0.05$) with males having higher GSI values than females. Males sampled in 2007 had significantly smaller GSI values than those sampled in 2009 (Tukey-Kramer HSD, $\alpha = 0.05$). While GSI increases throughout the sampling period, it was only significantly different for some month comparisons

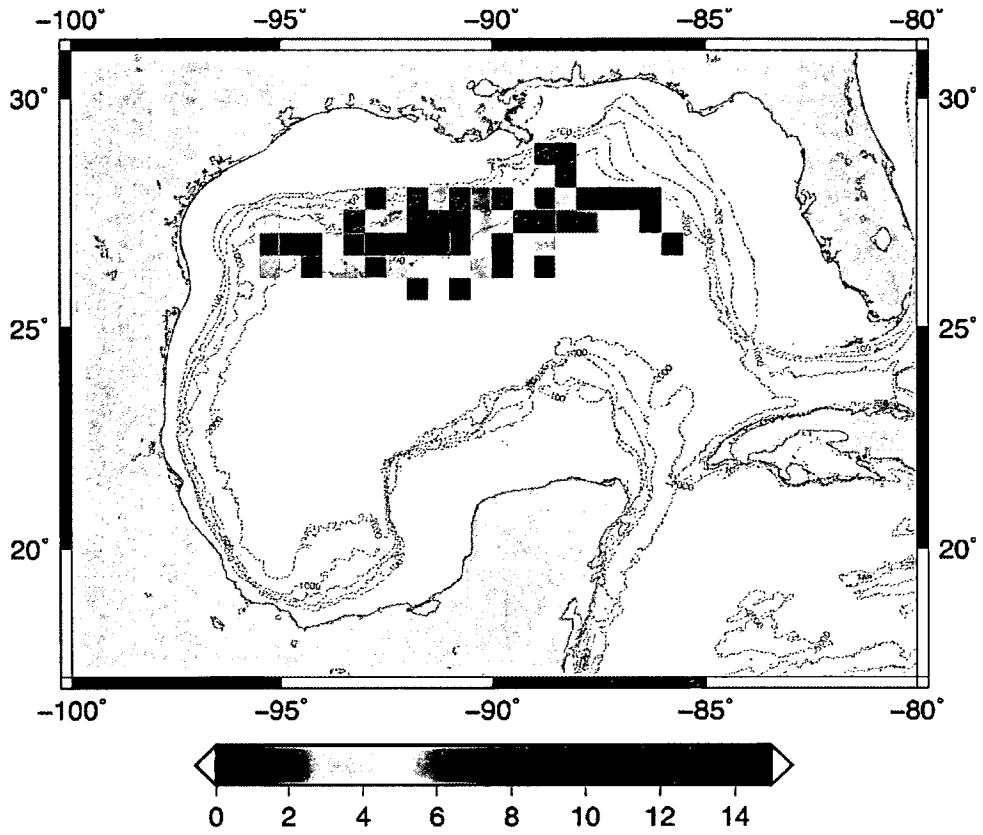

Figure 2-4. Map of the Gulf of Mexico showing the locations where Atlantic bluefin tuna, *Thunnus thynnus* were sampled by observers on commercial longliners for this study. According to NMFS privacy guidelines, exact locations have been aggregated.

(Table 2.5; Tukey-Kramer HSD, $\alpha = 0.05$).

Histology

Male classification

All stages of active spermatogenesis were observed in all samples collected from the Gulf of Mexico spawning grounds (Figure 2-7). While all samples were classified as spawning according to Santamaria et al. (2003) and Abascal et al. (2004), a few samples appeared to be post-spawning due to residual spermatozoa in the collecting duct (Figure 2-8). These post-spawning fish were still undergoing active spermatogenesis,

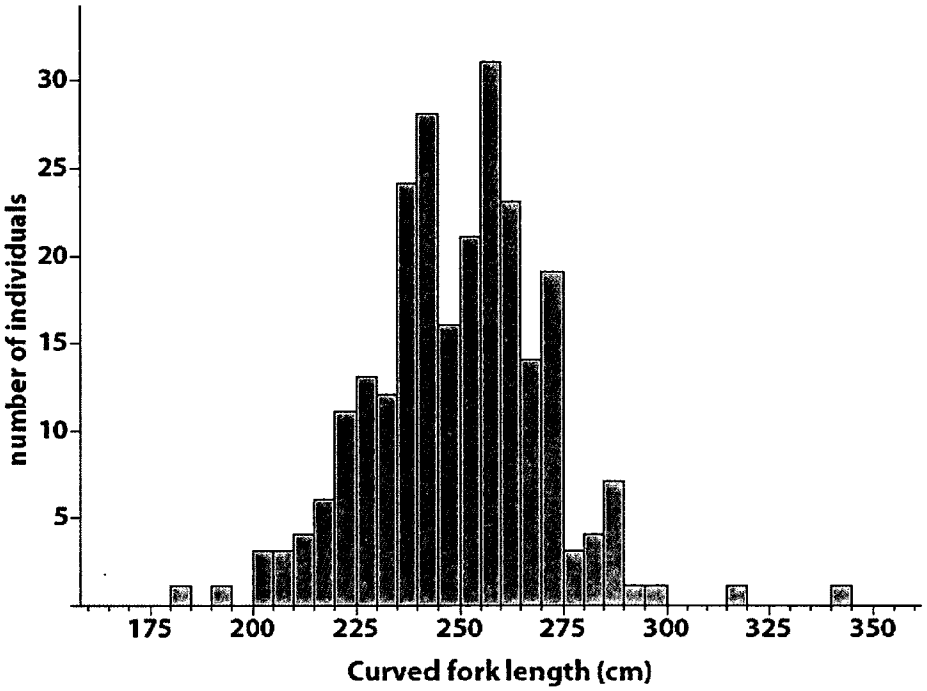

Figure 2-5. Length frequency distribution for Atlantic bluefin tuna, *Thunnus thynnus*, sampled from the Gulf of Mexico. All fish larger than 205 cm are 'giants.'

Sex		CFL (cm)	BW (kg)	GW (kg)	GSI
Male	$\mu(\pm SD)$	252.6(±20.9)	274.6(±72.3)	8.37(±3.52)	3.15(±1.29)
	Range	200–319	132.2–497.3	0.9–16.5	0.479–6.18
	n	92	93	75	75
Female	$\mu(\pm SD)$	246.8(±20.8)	253.7(±66.51)	7.0(±3.3)	2.73(±1.2)
	Range	180–341	98.6–582.4	1.1–16.9	0.350–7.25
	n	154	154	112	111

Table 2.4. Biometric data summarized for Atlantic bluefin tuna, *Thunnus thynnus*, sampled from the Gulf of Mexico between 2007 and 2009 for this study. CFL = curved fork length; BW = body weight; GW = gonad weight; GSI = gonadosomatic index.

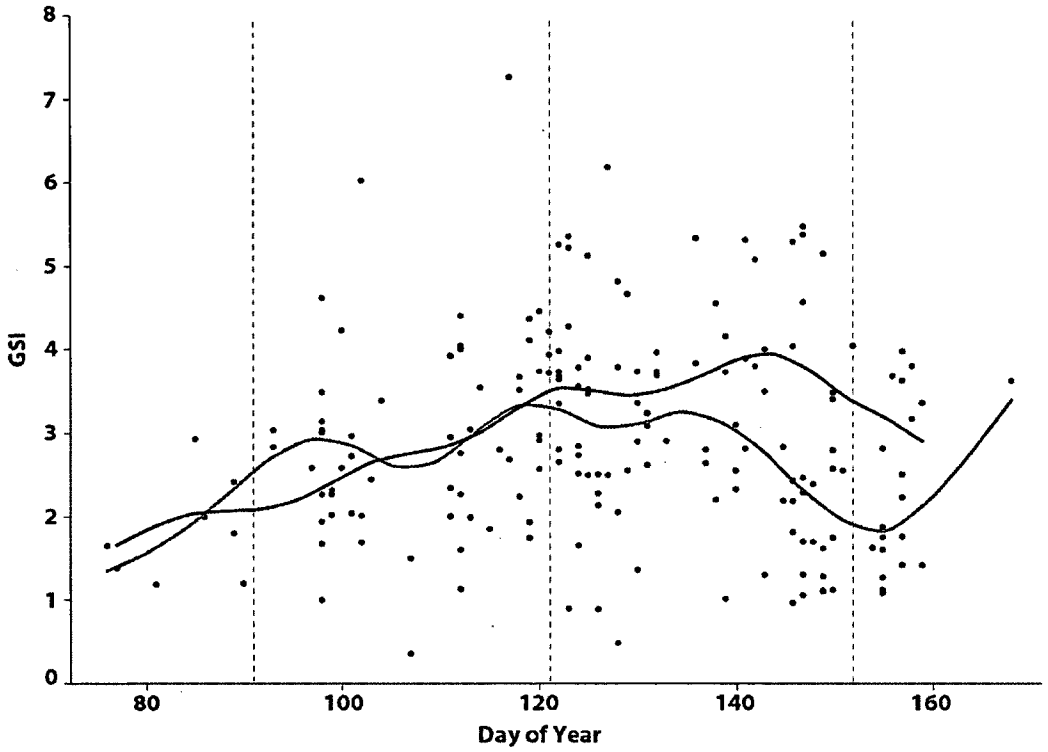

Figure 2-6. Gonadosomatic index (GSI) for Atlantic bluefin tuna, *Thunnus thynnus*, over the course of all sampling seasons. All years are aggregated, and the dotted lines indicate 1-April, 1-May, and 1-June. The smooth lines show the trend of GSI over time where blue is males and red is females. Males had significantly higher GSI values than females (student's t-test, $p < 0.05$).

Sex	Month			Mean GSI
Sexes pooled	3		b	1.8167
	4	a	b	2.7826
	5	a		2.9916
	6	a	b	2.4243
Male	3	a	b	1.9788
	4		b	2.6862
	5	a		3.5448
	6	a	b	3.2247
Female	3	a	b	1.6546
	4	a		2.8937
	5	a		2.7356
	6		b	1.8241

Table 2.5. Mean gonadosomatic index (GSI) values for males and females by month. Rows separated by different letters are significantly different (Tukey-Kramer HSD, $\alpha = 0.05$).

and had some spermatozoa in the ducts, and thus, were still classified as spawning as opposed to regressed.

Female classification

Bluefin tuna ovaries develop asynchronously, thus oocytes in various stages of development are simultaneously found in the ovary (Figure 2-9). In our samples, all stages of oocyte development were observed (Figure 2-10).

There was no significant difference in the gonad development between years, so samples were pooled across years for analyses. The number of inactive (both mature and immature) females ranged between 20% and 30%. The proportion of active non-spawning (ANS) females was 18.5% in 2007, 28.3% in 2008 and 25.0% in 2009. Proportions of active spawning (AS) females remained relatively consistent across years (approximately 40–50% of the females; Figure 2-11).

When ovary samples were arranged by month, consistent differences were observed in maturity stages between months with increasing maturation throughout the sam-

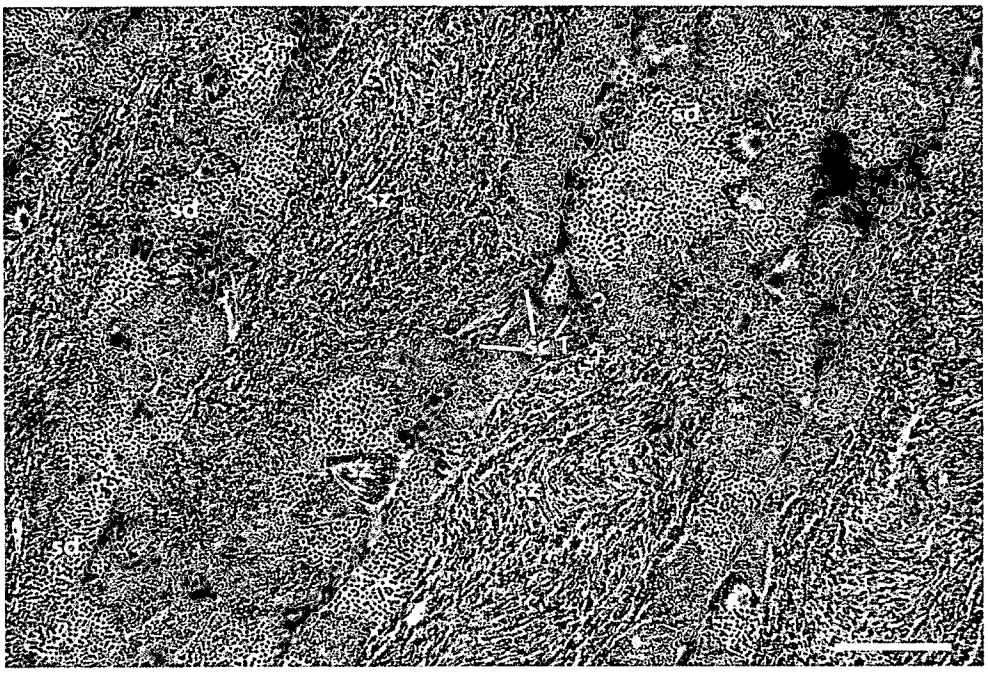

Figure 2-7. Stages of spermatogenesis in Atlantic bluefin tuna, *Thunnus thynnus*, sampled from the Gulf of Mexico: sc 1, primary spermatocytes; sd, spermatids; sz, spermatozoa; CFL = 236 cm, BW = 229 kg. Scale bar is 100 μm.

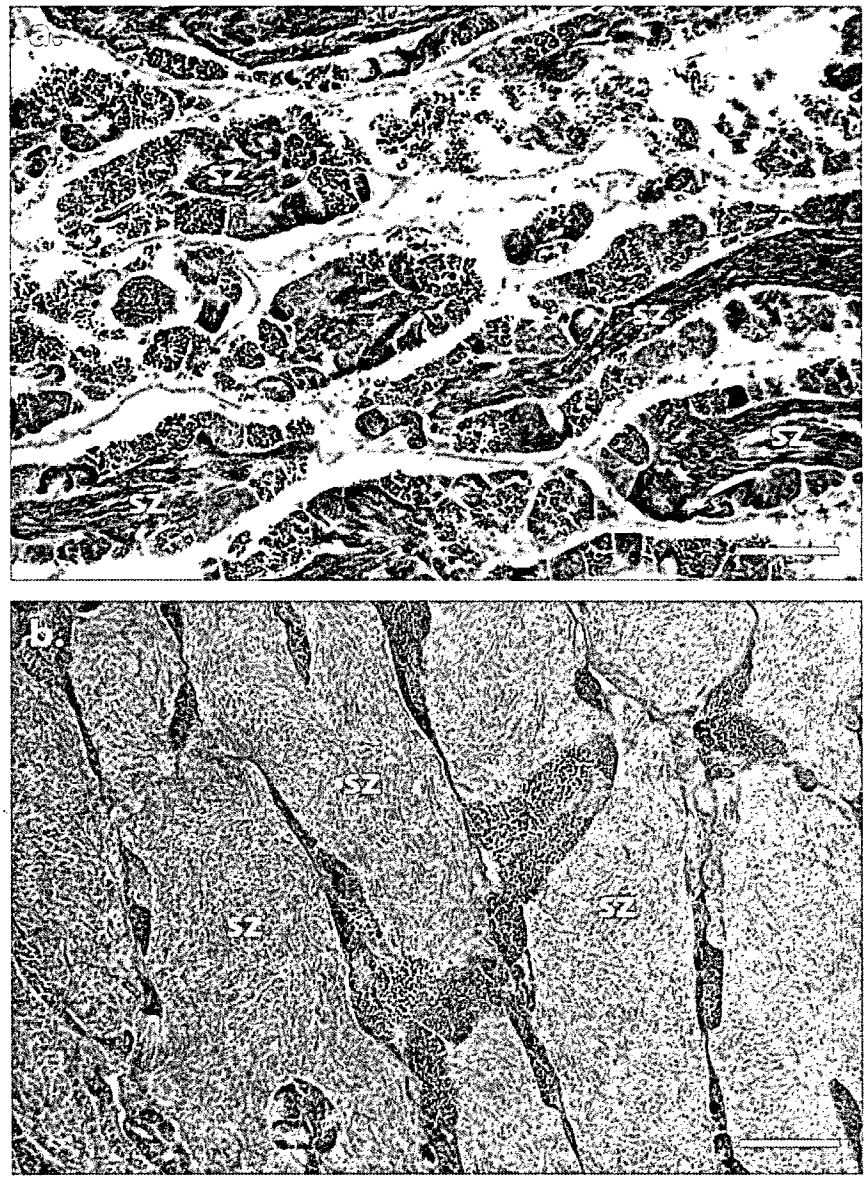

Figure 2-8. A comparison of post-spawning (A) and active spawning (B) testes from Atlantic bluefin tuna, *Thunnus thynnus*, sampled from the Gulf of Mexico. In the post-spawning individual (A), some spermatozoa are still present, but the ducts are not nearly as full as in the active spawning fish (B). A: CFL = 261 cm, BW = 382 kg. B: CFL = 265 cm, BW = 313 kg. Scale bar is 100 μm.

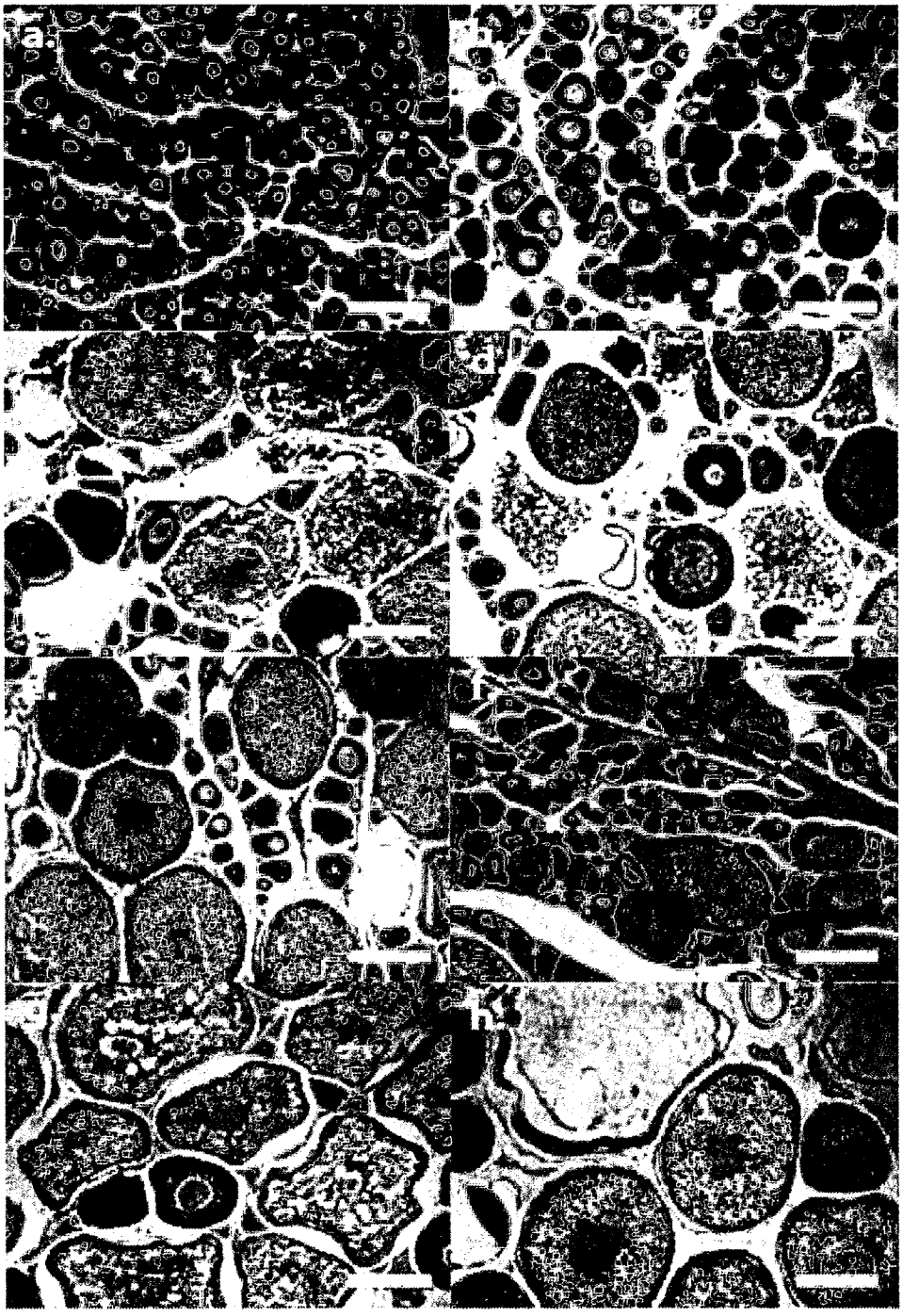

Figure 2-9. Maturity stages assigned to Atlantic bluefin tuna, *Thunnus thynnus*: (a,b) Inactive, immature (II); (c,d) Inactive, mature (IM); (e,f) Active, non-spawning (ANS); (g,h) Active, spawning (AS). Scale bar is 250 μm for all images.

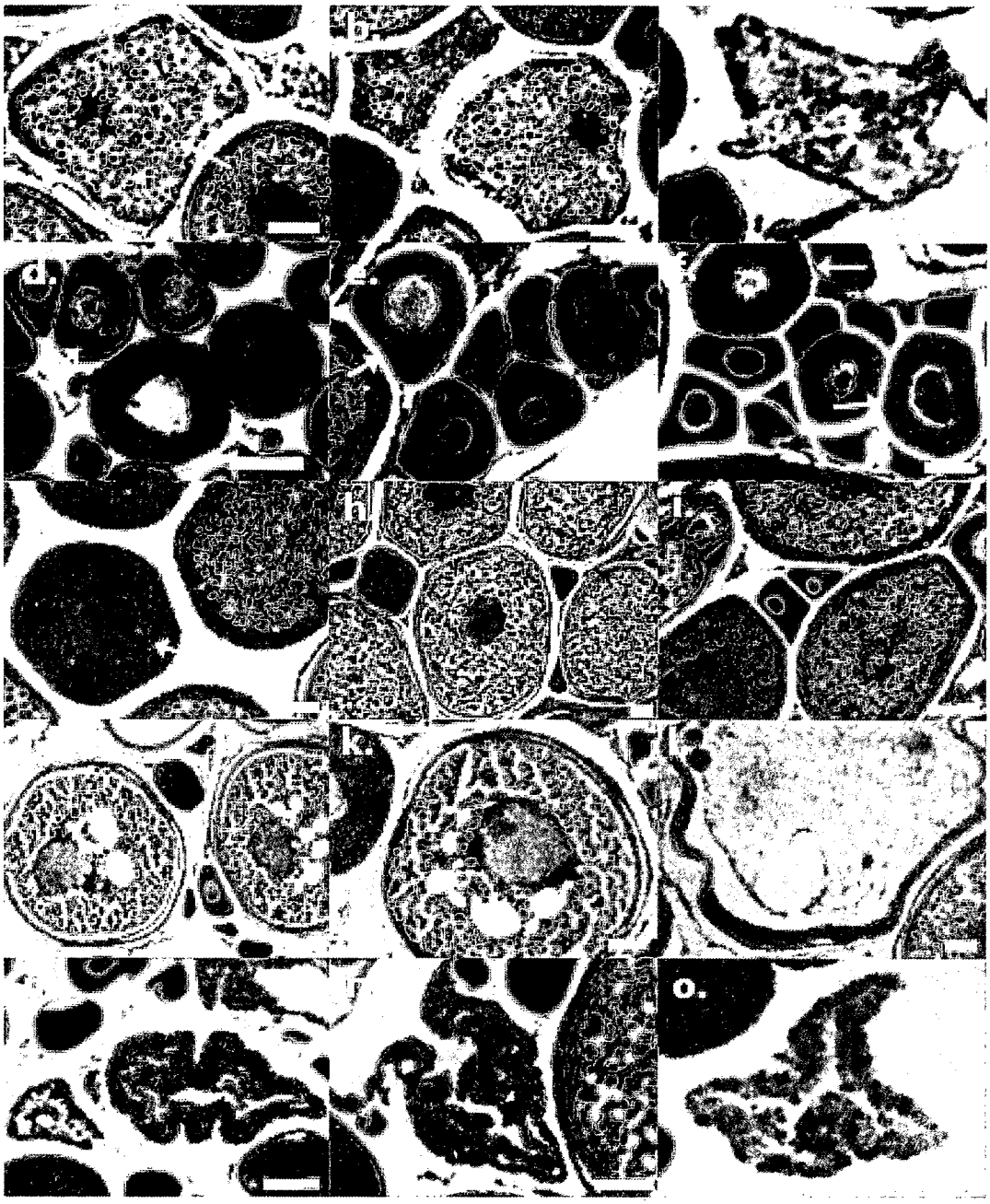

Figure 2-10. Oocyte development stages from Atlantic bluefin tuna, *Thunnus thynnus*: (a,b) α atresia (arrows); (c) β atresia; (d) early lipid stage follicle (arrow) next to later stage lipid follicle; (e,f) lipid stage follicles (arrow); (g-i) vitellogenic follicles with early stage vitellogenic marked with an arrow; (j,k) migratory nucleus oocytes; (l) hydrated oocyte; (m) side by side comparison of an old post-ovulatory follicle (POF; arrow) next to a newer POF; (n) very recent POF, not yet closed; (o) POF. Scale bar is 100 μm for all images.

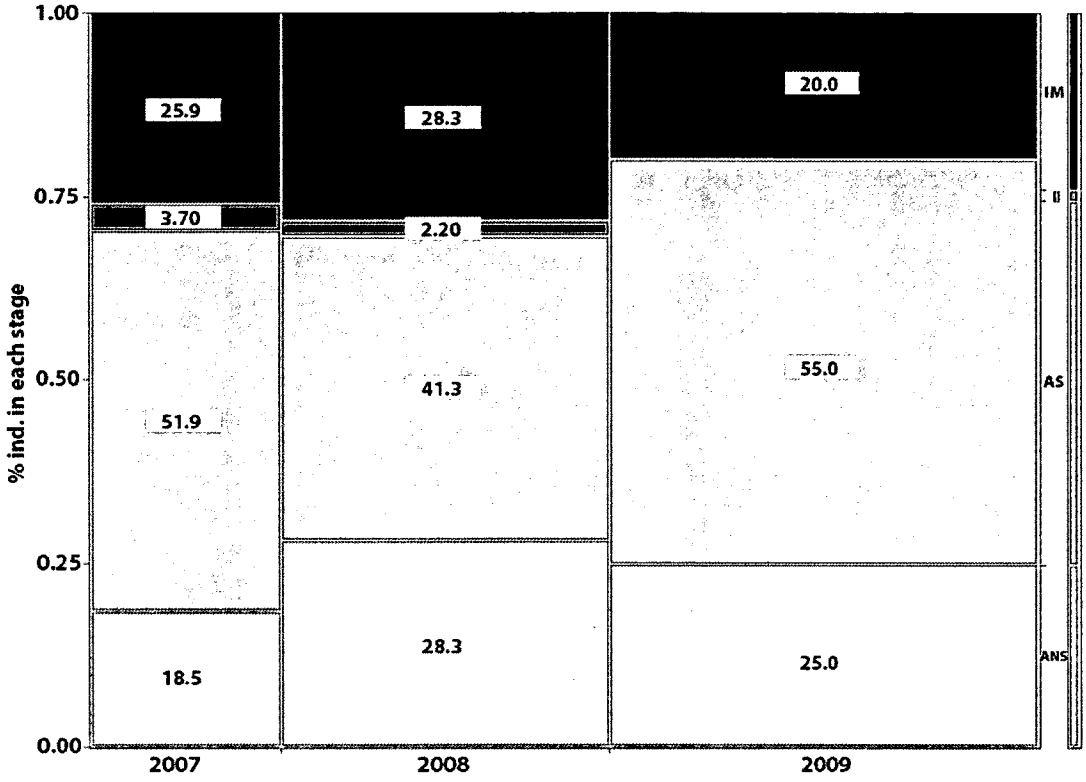

Figure 2-11. Percent of female Atlantic bluefin tuna, *Thunnus thynnus*, classified in each stage of reproductive maturity in each year. No statistical difference was found between years for each stage. Column widths are representative of sample size (2007 n=27; 2008 n=46; 2009 n=60). IM=inactive, mature; II=inactive, immature; AS=active spawning; ANS=active, non-spawning.

Month	Total	AS	With POFs	Spawning frequency		Spawning interval
				(Total)	(AS only)	(days)
April	30	12	9	0.30	0.75	3.33
May	68	45	35	0.51	0.78	1.94
June	10	7	5	0.50	0.71	2.00
all months	108	64	49	0.45	0.77	2.20

Table 2.6. Spawning frequency and spawning interval estimates for Atlantic bluefin tuna, *Thunnus thynnus*, sampled from the Gulf of Mexico. AS=active, spawning.

pling period (Figure 2-12). The samples collected in February and March did not include any AS females, and the proportion of AS females increased throughout the sampling period until a small decrease was observed in June.

Spawning frequency was estimated to be 0.45 throughout the sampling period as 49 out of 108 mature females contained POFs in their ovaries. When this parameter was calculated by month, the proportion of females with POFs caught in April was lower than that in May and June. When the spawning frequency was calculated considering only AS females, the proportion of spawning females increased significantly and remained similar among months (Table 2.6).

As POFs in bluefin tuna are reabsorbed within 24 hours of spawning, stereological counts of these particles provide an effective estimation of the realized batch fecundity. The total mean number of POFs (±SD) estimated for fish sampled in the Gulf of Mexico was $7.65 \cdot 10^6(\pm 6.71 \cdot 10^6)$, which corresponds to a relative batch fecundity (Ng^{-1}) of 28.14(±26.90) POF g^{-1} body weight.

Age Determination

Ages were estimated from the straight fork length (n=246; Eqn 2.1), total body weight (n=127), and by counting annual growth rings on dorsal spine sections (n=165). For ages estimated from BW, only fish that were weighed were used for this age estimation (i.e., fish were not aged based on weights estimated from length). Based

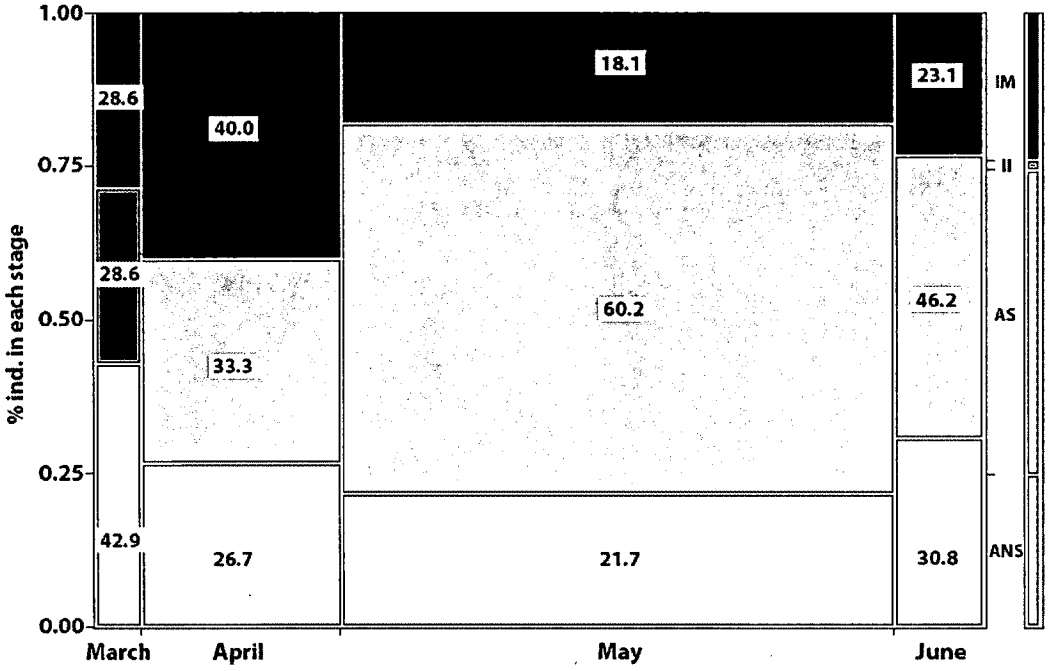

Figure 2-12. Percent of female Atlantic bluefin tuna, *Thunnus thynnus*, classified in each stage of reproductive maturity by month. Differences observed in maturity stages between months were consistent with the progression of the spawning season. The one sample collected on 28 Feb 2007 was included in the March samples. Column widths are representative of sample size (March n=7; April n=30; May n=83; June n=13). IM=inactive, mature; II=inactive, immature; AS=active spawning; ANS=active, non-spawning.

Aging method	n	minimum	maximum	mean	median
Dorsal spines	163	8	20	14	14
Body weight	127	10	35	16	16
Straight fork length	247	7	35	15	14

Table 2.7. Ages calculated using three different aging methods for Atlantic bluefin tuna, *Thunnus thynnus*, captured from the Gulf of Mexico spawning grounds. Annuli were counted on dorsal spines, and for body weight and straight fork length, ages were estimated according to Restrepo et al. (2010).

on the estimation of age from straight fork length or total body weight, bluefin tuna sampled for this study ranged in age from 7 years to over 35 years. Some fish were longer or heavier than those reported in Restrepo et al. (2010), and thus, an accurate age estimation was not possible.

A total of 168 dorsal spines were measured and sectioned for age analysis, and poor ring quality reduced the number of usable spines to 165 individuals. Using dorsal spines for aging is dependent on a strong linear relationship between dorsal spine diameter and SFL, and linear regression yielded an unexpectedly weak correlation for our sample (Figure 2-13). The length data received from the observers varied widely in the method of measurement (i.e., curved vs. straight fork length, fin length, etc.) and was not always labeled. We suspect this as the cause for such a weak correlation. Previous age and growth studies have shown a strong linear relationship for bluefin tuna length and dorsal spine diameter (Cort, 1991; Megalofonou & De Metrio, 2000; Golet, 2010), so despite the weak correlation, ages were still estimated from the dorsal spine sections. Based on the counts of translucent annuli, bluefin tuna sampled for this study ranged in age from 8 to 20 years.

When comparing all three aging techniques, the mean age ranged from 14-16 years depending on the method used (Table 2.7). The use of dorsal spines greatly limited the upper range of the ages (Figure 2-14).

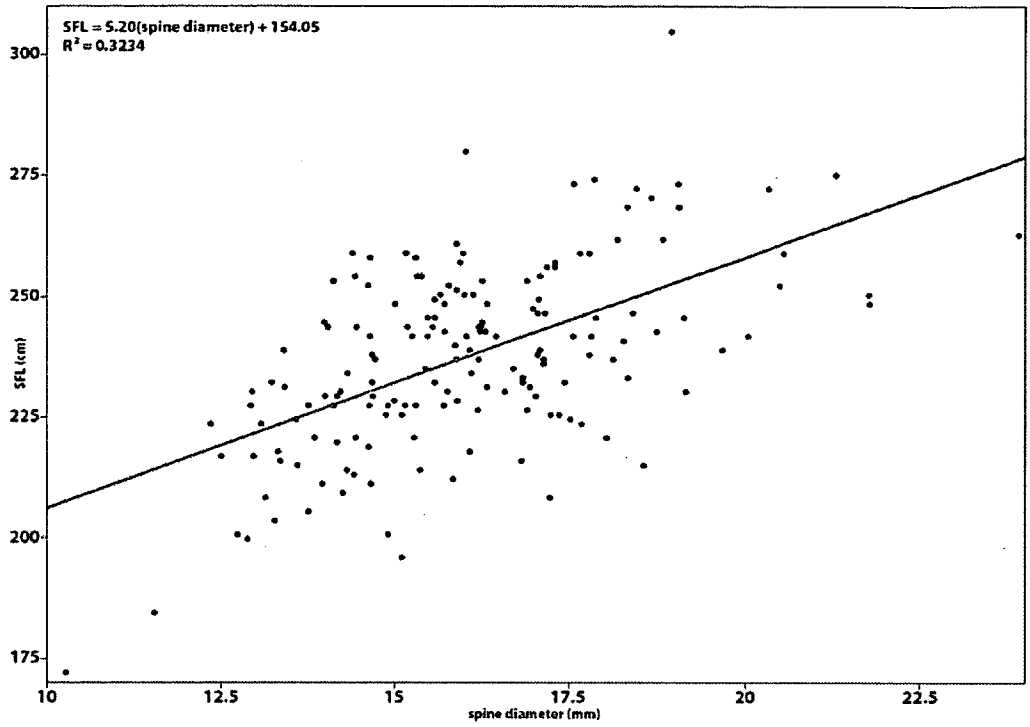

Figure 2-13. Relationship between maximum dorsal spine diameter and SFL for Atlantic bluefin tuna, *Thunnus thynnus*, sampled in the Gulf of Mexico between 2007–2009.

Discussion

Although not spatially or temporally exhaustive, this study represents the first attempt to use quantitative methods to assess the spawning condition of Atlantic bluefin tuna sampled from the Gulf of Mexico spawning grounds. Spatially and temporally comprehensive sampling of gonad tissue from the spawning grounds is required for evaluating the reproductive condition and performance of bluefin tuna. Additionally, systematic sampling on the spawning grounds allows the study of temporal variation in key reproductive parameters, such as sex ratio, proportion of mature fish, spawning frequency, and spawning periodicity. This information was lacking for the western stock resulting in large uncertainties for stock assessment and evaluation of produc-

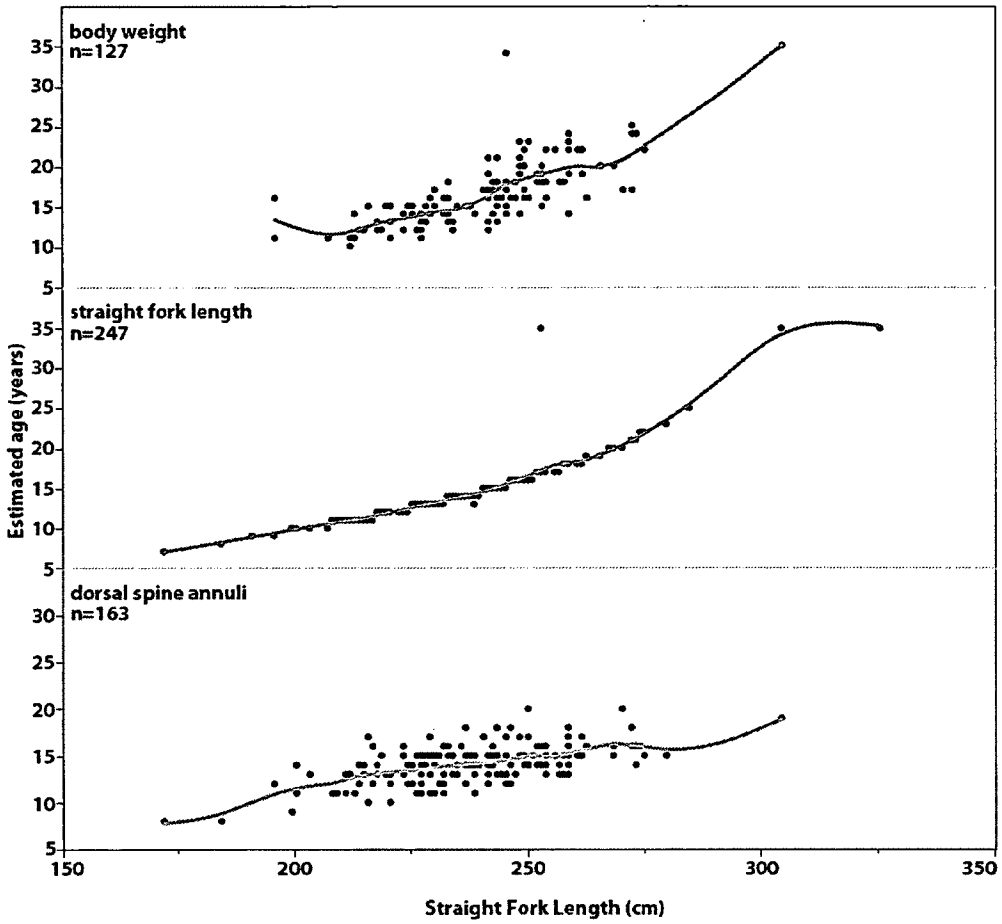

Figure 2-14. Ages calculated from straight fork length, body weight, and direct dorsal spine reading for Atlantic bluefin tuna, *Thunnus thynnus*, sampled on the Gulf of Mexico spawning grounds. Note the scale on the age from dorsal spines is slightly contracted to allow better visualization of the data spread.

tivity (Fromentin & Powers, 2005). Since the implementation of the moratorium on directed fishing for bluefin tuna in the Gulf of Mexico, federal fisheries observers have sampled bluefin tuna caught as bycatch in the yellowfin tuna and swordfish longline fisheries (*Thunnus albacares* and *Xiphius gladius*, respectively). Such restrictions prohibit comprehensive size sampling of spawning bluefin tuna in the Gulf of Mexico and hinder the determination of the spatial and temporal extent of western spawning. As a result of the bycatch sampling, previous studies have lacked small/medium fish (<180 cm) leading to larger/older size and age at maturity estimates (Diaz & Turner, 2007; Diaz, 2011). Additionally, despite the lack of spatially comprehensive sampling, these recent studies assumed bluefin tuna only spawn in the Gulf of Mexico and based the age at maturity estimate on age estimations from the length of fish collected as bycatch.

Goldstein et al. (2007) suggested previous bluefin tuna sampling did not accurately represent the spawning size range of the western population because it only included fish sampled by longliners on known spawning grounds rather than all size classes sampled throughout their range. Gear type, size selectivity, and vertical distribution of tuna by size also influence the size of spawners sampled by commercial fishing fleets (Davis & Farley, 2001; Medina et al., 2007). As yellowfin tuna caught by longline fishing gear are, on average, 120 cm (Schaefer, 1998), longline fishing vessels in the Gulf of Mexico would be expected to capture bluefin tuna of similar size; however, prior to the US moratorium, catch records indicated the presence of only giant bluefin tuna (>205 cm, CFL) in the Gulf of Mexico (Mather et al., 1995). As opposed to the current management paradigm of western bluefin tuna maturing at an older age than the eastern stock, fish may exhibit size and temporal segregation on the spawning grounds as is seen with the eastern spawning stock (Mather et al., 1995; Lutcavage et al., 1999; Heinisch et al., 2008) and in Pacific bluefin tuna (*T. orientalis*; Itoh,

2006). There is indirect evidence that smaller fish utilize alternate locations, such as the Caribbean Sea, the Bahamas, or the Gulf Stream margins because ripe fish have been collected there (Rivas, 1954; Wilson & Bartlett, 1967; Mather et al., 1995). Given this evidence, it is possible that smaller bluefin tuna spawn in alternative locations within the Gulf of Mexico as previous sampling has been concentrated in the north/central Gulf where US longline vessels operate. An Atlantic bluefin tuna life history model predicts smaller/younger maturing fish should have shorter migration routes and spawn in areas closer to feeding areas than larger/older fish with high energy reserves (Chapman et al., 2011).

The sex ratio of female to male fish sampled for this study was 1.6:1, and males were, on average, heavier than females, a result found previously for bluefin tuna (Baglin, 1980, 1982) and yellowfin (Schaefer, 1998). Skewed sex ratios have been previously reported for bluefin tuna with more females present in southern sampling locations (Rivas, 1976) and more males present in northern sampling locations (Caddy & Butler, 1976).

In this study, the smallest female sampled was 180 cm, and the smallest male was 200 cm. According to recent age and growth studies, these fish are between 7–8 years old (Santamaria et al., 2009; Restrepo et al., 2010), and our dorsal spine readings are consistent with these estimations for these fish. The female fish had mature ovaries (Figure 2-1) containing advanced stage vitellogenesis and numerous recent POFs indicating recent spawning. The male sample had active spermatogenesis and collecting ducts full of spermatozoa indicating imminent spawning. These findings do not support the ICCAT recognized age at 100% maturity of 9 years and do not support recent suggestions based on catch data to increase the age at maturity for western fish to 12–16 years (Diaz, 2011; Teo et al., 2007a). While the majority of our fish were between 13 and 18 years old, this does not indicate only older fish are actively

spawning in the western stock as the potential for size segregation and/or alternative spawning locations exists. Historical papers indicate potential spawning areas further south in the Florida Straits and outside the Gulf of Mexico in the Caribbean (Rivas, 1954; Mather et al., 1995).

Electronic tagging studies have shown fish of assumed reproductively mature size outside either known spawning area during the presumed spawning season (Lutcavage et al., 1999; Galuardi et al., 2010) but no histological sampling has been conducted to confirm the reproductive status of these fish. In a recent electronic tagging study examining migration patterns of juvenile bluefin tuna (2–5 years), no fish entered the Gulf of Mexico during the spawning period (April–May); however, there was an aggregation of fish north of the Bahamas and in the mid-Atlantic Bight (Galuardi & Lutcavage, 2012). In order to gain a more realistic view of the population dynamics of the western stock and resolve the age at maturity paradox, further reproductive studies must include gonad sampling for histology in these areas during the months that juvenile bluefin tuna are present.

Previous reproductive studies on western spawning bluefin tuna have focused primarily on females (Baglin, 1976, 1982; Diaz & Turner, 2007; Goldstein et al., 2007; Diaz, 2011), while sampling male fish has proven to beneficial for determining maturity and reproductive status in eastern bluefin tuna and other fish species (Abascal et al., 2004; Maki Jenkins & McBride, 2009). All males sampled from the Gulf of Mexico contained spermatozoa in the collecting duct and were undergoing active spermatogenesis in the testis periphery. This is typical of actively spawning fish and is expected for fish sampled from the spawning grounds. As males do not reabsorb unused spermatozoa, sampling smaller males away from the spawning grounds may help elucidate the age at maturity for bluefin tuna.

The histological examination of bluefin tuna ovaries showed a progression of follicle maturation consistent with what would be expected as the spawning period advances. While samples collected in February and March contained no active spawning (AS) individuals, 33% of samples collected in April were AS. As the spawning season progressed, the number of AS females increased and peaked in May at 60%. GSI was significantly different between months with the levels measured in March and June being the lowest. This is consistent with smaller gonad size associated with the beginning and end of the spawning season. We also found the male fish had a significantly higher GSI than the female fish but there was no significant difference in body weight between males and females. This indicates that males in our sample had larger gonads than females, which is atypical for fish (Belle, 1995) but is likely due to the highly selective nature of the sampling method. Our data show the beginning of an increase in female GSI at the end of the sampling period (Figure 2-6), but this is likely due to one large fish with a high GSI and not representative of a trend in GSI increase.

The realized fecundity calculated here (28.14 eggs g^{-1}) is lower but not significantly different than that previously calculated for eastern spawning bluefin tuna (48.22 eggs g^{-1} Aranda et al., 2012). This discrepancy in fecundity could be due to the restricted spatial and temporal sampling conducted in the Gulf of Mexico or could be a result of the sampling gear used.

While we sampled only one fish caught in February, it is worth noting that this fish measured 245 cm and weighed 191 kg. No dorsal spine was collected from this fish, but according to ICCAT growth curves, this fish was about 14 years old (Restrepo et al., 2010). Its ovarian histology showed almost entirely primary growth oocytes with very few lipid stage oocytes and no indication of further oocyte maturation (Figure 2-15). It is possible that this fish is skipping spawning, or oocyte maturation in bluefin tuna may take two months or less. An accurate timeline for bluefin tuna

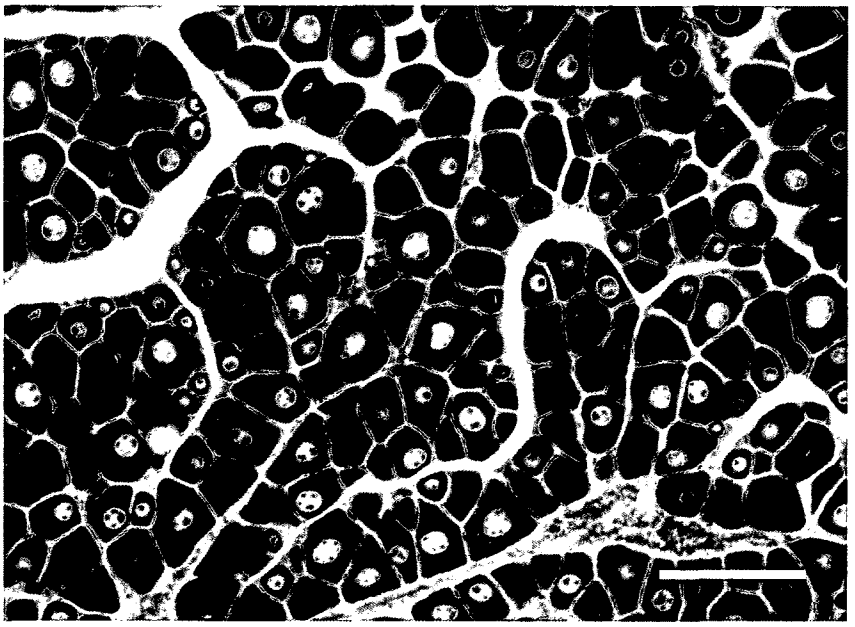

Figure 2-15. Micrograph of ovarian tissue collected from an Atlantic bluefin tuna, *Thunnus thynnus*, collected from the Gulf of Mexico on 28 February 2007. Note all primary growth oocytes with no indication of further oocyte maturation. Scale bar is 250 μm.

oocyte maturation from primary growth oocytes to fully hydrated oocytes has not been determined. Based on studies of southern bluefin tuna, final oocyte maturation (from vitellogenic to hydrated) is believed to occur in less than one day (Farley & Davis, 1998). In our samples, fish sampled in mid-March (just over 2 weeks later) contained early to late vitellogenic oocytes indicating ongoing oocyte maturation. March samples also contained both $\alpha-$ and $\beta-$atresia indicating some oocyte reabsorption. The uncertainty surrounding the time required for oocyte maturation warrants further exploration and indicates the need for more comprehensive spatial and temporal sampling of gonad tissue for histology.

2.2 Atlantic bluefin tuna sampled from the Bahamas

Introduction

Historic studies note Atlantic bluefin tuna spawning outside the Gulf of Mexico and Straits of Florida including the Bahamas and other Caribbean locations (Rivas, 1954; Mather et al., 1995). Nonetheless, contemporary studies of western bluefin tuna have focused almost exclusively on the Gulf of Mexico and have not utilized direct examination of gonad tissues (Diaz & Turner, 2007; Diaz, 2011). Bluefin tuna larvae have been collected outside the Gulf of Mexico and Straits of Florida suggesting alternative spawning grounds may exist (McGowan & Richards, 1989; Muhling et al., 2011a). Additionally, electronic tagging studies have shown both presumed immature (ages 2–5 years) and mature fish ($\geq$8 years) outside the known spawning areas during the presumed spawning season (Lutcavage et al., 1999; Block et al., 2005; Sibert et al., 2006; Galuardi et al., 2010) with some fish remaining near the Bahamas and south of the mid-Atlantic bight (Galuardi & Lutcavage, 2012).

Here, we use histology to describe the maturity and reproductive status of fish sampled by longline fishing from the Bahamas, an area not currently recognized as a western bluefin tuna spawning ground. Previous studies of fish sampled from the Bahamas have relied on macroscopic gonad examination and/or overall fish condition (Rivas, 1955). This is the first time direct histological analyses have been conducted on fish sampled from the Bahamas.

Methods

Gonads were collected from Atlantic bluefin tuna caught on commercial longline fishing vessels around the Bahamas during the presumed western spawning season (April–May). Four of the fish (sample ID's 4, 13, 15, and 17) were collected by LPRC scientists, and the remaining eight fish were collected by the fishing vessel's

captain or crew members. Curved fork length was measured on board and converted to straight fork length (SFL) for age and weight estimations (Eqn. 2.1). Body weight (BW) was estimated from either dressed weight (DW; Eqn 2.2) or from SFL according to ICCAT conversion factors (Table 2.1). Gonads were weighed on board, and the gonadosomatic index was calculated (Eqn. 2.3).

Whole gonads were dissected from the body cavity immediately upon capture and weighed to the nearest kilogram. Sex was determined by macroscopic examination of the gonads (Figure 2-1). Subsamples were excised from the middle of the gonad and fixed in 10% neutral buffered formalin within 24 hours of collection. Tissue samples were rinsed and stored in 70% ethyl alcohol (EtOH), dehydrated in a series of increasing concentrations of EtOH, and cleared with ClearRite3®. Tissue samples were embedded in paraffin wax, sectioned to 5 μm sections, stained with haematoxylin and eosin, and mounted on glass slides using a high clarity mounting medium. Maturity status for was determined by examining the entire slide using a compound microscope (40–100x). Females and males were classified according to Schaefer (1998) and Santamaria et al. (2003), respectively, as previously described (Tables 2.2 and 2.3).

Results

In April–May 2012, twelve bluefin tuna were sampled from commercial longline fishing vessels operating in the Bahamas (Figure 2-16). The sex ratio was skewed with eight females and four males sampled. GSI was 0.86–5.73 though because gonad weight was not always measured, GSI was only calculated for four fish. All fish were presumably mature (i.e., >196 cm, CFL) and had estimated ages of 9–17 years ($\bar{x} \pm SD = 13.1 \pm 2.28$; Restrepo et al., 2010). Curved fork length (CFL) was 207–265 cm and body weight (BW) was 146–326 kg (Table 2.8). There was no significant

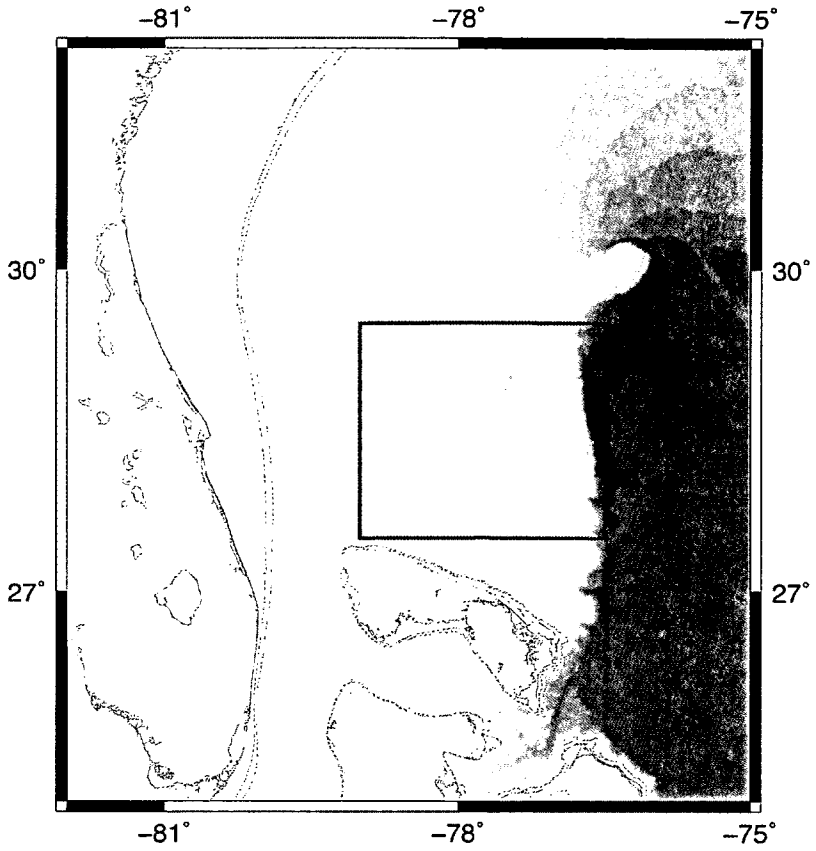

Figure 2-16. Map of the Bahamas showing the area where Atlantic bluefin tuna, *Thunnus thynnus* were sampled from commercial longline fishing vessels.

difference between males and females for either CFL or estimated BW (Tukey-Kramer HSD, $p = 0.7$ and $p = 0.63$, respectively), though our sample size is very small.

All males collected were in the spawning stage (Figure 2-17g,h), though one sample had residual spermatozoa in the ducts as opposed to full ducts (Figure 2-17f). Because active permeation was continuing in the periphery, this fish was still classified as spawning and not regressed.

Females were found in all reproductive stages except inactive immature (Figure 2-17). All active non-spawning (ANS) fish were pre-spawning with ovaries containing

Sample ID	Date	Sex	CFL (cm)	BW (kg)	GW (kg)	GSI	Maturity Stage
4	19-Apr	F	231	197.6	2.5	1.27	ANS
13	1-May	F	265	326.4*	18.7	5.73	ANS
15	3-May	F	245	229.3	7.9	3.45	ANS
17	3-May	M	237	209.9	1.8	0.86	Sp
30	-	M	-	249.8*	-	-	Sp
32	-	M	-	-	-	-	Sp
33	-	F	227	188.7	-	-	ANS
34	30-Apr	F	257	264.0	-	-	IM
35	-	F	255	260.4	-	-	AS
36	-	F	250	246.3	-	-	AS
37	4-May	F	207	145.5	-	-	ANS
38	4-May	M	226	185.8	-	-	Sp

Table 2.8. Biometric data for Atlantic bluefin tuna, *Thunnus thynnus*, sampled from the Bahamas by longline in April–May 2012. Body weights were estimated from either dressed weight (*) or straight fork length. Maturity stages for females and males were determined according to Schaefer (1998) and Santamaria et al. (2003), respectively. CFL=curved fork length, BW=body weight, GW=gonad weight, GSI=gonadosomatic index, AN=active non-spawning, IM=inactive mature, AS=active spawning, Sp=spawning.

early to late vitellogenic oocytes with little to no atresia (Figure 2-17a,b). Sample 34 was classified as inactive mature as it was long post-spawning with copies β-atresia and some γ-atresia (Figure 2-17e). Two females (samples 35 and 36) were classified as active spawning (AS) with ovaries containing late vitellogenic oocytes and migratory nucleus oocytes (Figure 2-17c,d). The number of migratory nucleus oocytes was minimal, but most vitellogenic oocytes were very progressed with large yolk and lipid droplets. These two samples contained minimal amounts of α-atresia.

Discussion

While not spatially or temporally comprehensive, this is the first histological examination of Atlantic bluefin tuna gonads collected from the Bahamas, a potential spawning location. Our sample size is very limited; however, our results present evidence that

further sampling in this region and other potential spawning areas outside the Gulf of Mexico is warranted. While we cannot be certain about the direction of travel, the fishermen were fishing on a northeastern trajectory and continued catching bluefin tuna, thus, they do not believe the fish were heading to the Gulf of Mexico (Capt. Bob Kane, pers. comm.).

The sex ratio of females to males was 2:1, and skewed sex ratios with more females have previously been reported for bluefin tuna sampled in southern regions (Rivas, 1976). All the males sampled here contained spermatozoa in the ducts and had active spermatogenesis in the periphery. This is typical of actively spawning fish and is expected for fish sampled on spawning grounds. Most females were classified as active non-spawning and appeared to be pre-spawning with early vitellogenic oocytes and limited atresia. It is possible these fish could be moving towards the Gulf of Mexico to spawn, but it is also possible they could be spawning in the Caribbean or further north as historically reported (Rivas, 1954; Wilson & Bartlett, 1967; Mather et al., 1995).

The rates of oocyte maturation, post-ovulatory follicle degeneration, and oocyte atresia are temperature dependent and more rapid in tunas than in fish in cooler waters (Fitzhugh & Hettler, 1995). As oocyte maturation (from vitellogenic to hydrated) is believed to occur in less than one day for tunas (Farley & Davis, 1998), the two fish we sampled with migratory nucleus oocytes could be expected to spawn within 24h. Even if these fish were moving towards the Gulf of Mexico, it is unlikely they could swim to the north/central region of the Gulf of Mexico, the known western spawning location, in one day as travel rates are 4–8 kts (Lutcavage et al., 2000). Additionally, using mark recapture data, Mather et al. (1995) showed bluefin tuna making trans-Atlantic migrations at a rate of about 6 kts.

The one female classified as inactive mature (IM) contained extensive β-atresia. Hunter & Macewicz (1985) reported rates of atresia for starved northern anchovy (*Engraulis mordax*) as about three weeks from no atresia to β-atresia. It is possible this fish spawned in the Gulf of Mexico and had begun the northern migration to the foraging grounds.

Despite historical evidence of bluefin tuna spawning outside the Gulf of Mexico (Rivas, 1954; Wilson & Bartlett, 1967; Mather et al., 1995) and larval collections outside this known spawning area (McGowan & Richards, 1989; Muhling et al., 2011a), spatially and temporally comprehensive sampling of the western spawning stock is lacking. These data represent the first direct histological examination of bluefin tuna collected in the Bahamas, but with such a limited sample size, caution should be applied in interpreting the condition of these fish. However, because we found females in advanced stages of oocyte maturation, more sampling in this location and other potential alternative spawning grounds (Lutcavage et al., 1999; Galuardi et al., 2010) is necessary to understand the reproductive potential of western bluefin tuna (Fromentin & Powers, 2005; Takeuchi et al., 2008).

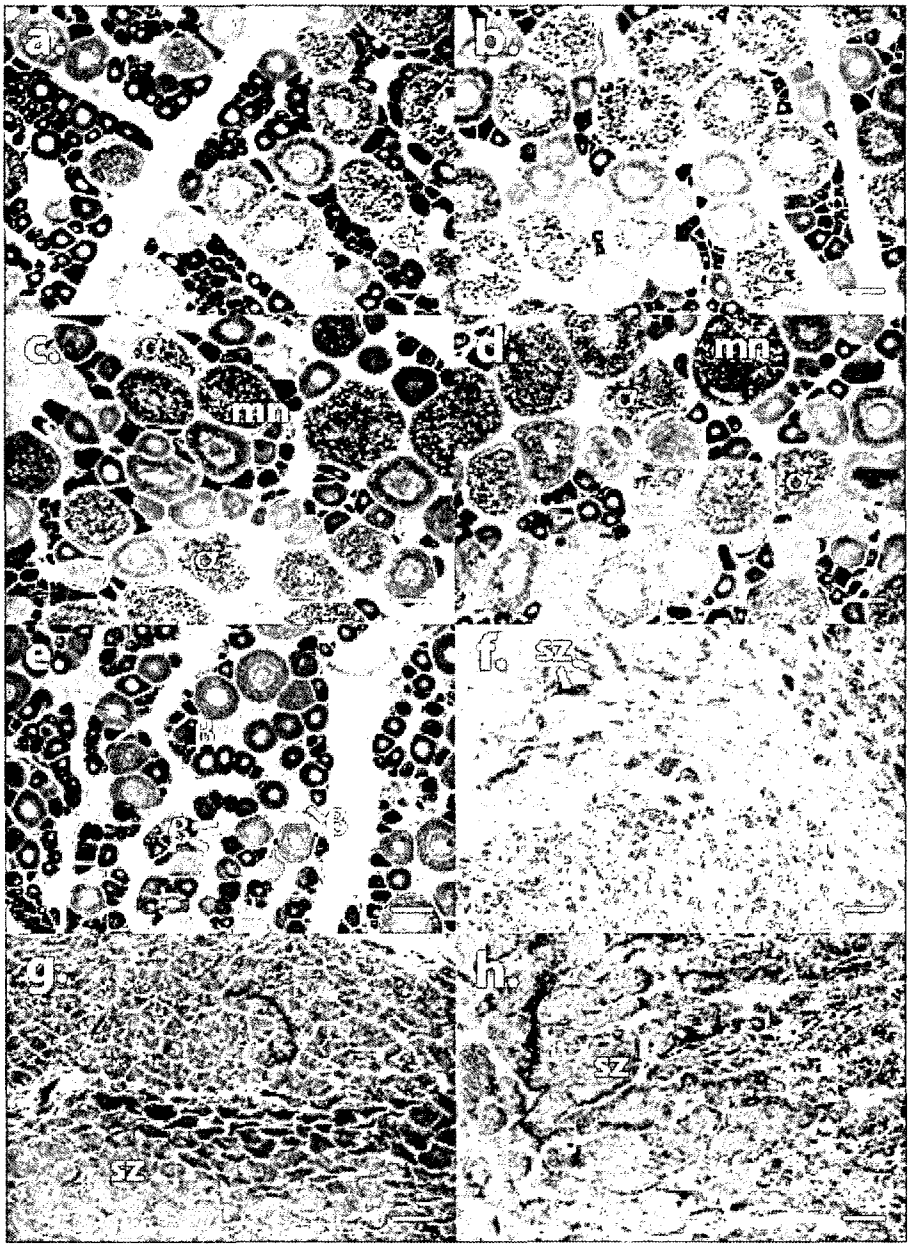

Figure 2-17. Maturity stages observed in Atlantic bluefin tuna, *Thunnus thynnus*, collected from the Bahamas by longline in April–May 2012. (a,b) active non-spawning females, (c,d) active spawning females, (e) inactive mature female, (f) spawning male with residual spermatozoa, (g,h) spawning males with active spermatogenesis and ducts full of spermatozoa. α=α-atresia, mn=migratory nucleus, β=β-atresia, sz=spermatozoa. Scale bar is 200 μm.

CHAPTER 3

COMPARATIVE HISTOLOGICAL AND STEREOLOGICAL ASSESSMENT OF THE REPRODUCTIVE STATUS OF FEMALE ATLANTIC BLUEFIN TUNA FROM THE GULF OF MEXICO AND THE MEDITERRANEAN SEA

Introduction

The reproductive biology of Atlantic bluefin tuna (*Thunnus thynnus*, L. 1758) remains poorly understood despite the high economic value of this fishery and its exploitation throughout the Atlantic Ocean and the Mediterranean Sea. The two spawning stocks (eastern and western) spawn in the Gulf of Mexico and the Mediterranean Sea, and this division assumes a low level of mixing between the stocks, separate spawning grounds, and spawning site fidelity.

The Tyrrhenian Sea, the Levantine Sea, and the Balearic Sea have been identified as localized spawning grounds in the Mediterranean Sea (Dicenta, 1977; Corriero et al., 2003; Oray & Karakulak, 2005). Gonad histology studies have identified the eastern spawning season as mid-May through mid-July (Medina et al., 2002, 2007; Corriero et al., 2005; Heinisch et al., 2008). Larval surveys (Richards, 1976; Montolio & Juarez, 1977) and macroscopic ovary examinations (Rivas, 1954; Baglin, 1982) have identified the central Gulf of Mexico and the Florida Straits as spawning areas for the western stock. According to histological analyses of oocyte maturation, the western spawning season runs from April to June with a peak in May (Rivas, 1954; Baglin, 1982). Although these earlier studies provide valuable information, a comparative examination of the reproductive biology of both spawning stocks is lacking. As the

eastern stock is larger than the western stock, the mixing rates between the two stocks are unbalanced with the eastern stock having greater influence on the western population; consequently, any management action aimed at the eastern stock may indirectly affect the western stock (Rooker et al., 2008a). Therefore, more research on the reproductive potential of both stocks is necessary as their reproductive potential influences recruitment and hence the sustainability of the stocks and their capacity for supporting commercial fisheries (Baglin, 1982; Mather et al., 1995).

Extensive research has been conducted on the reproductive biology and reproductive potential of the eastern stock (Susca et al., 2001a; De Metrio et al., 2002; Medina et al., 2002, 2007; Mourente et al., 2002; Santamaria et al., 2003; Viñas et al., 2003; Corriero et al., 2003, 2005; Karakulak et al., 2004b; Oray & Karakulak, 2005; Aranda et al., 2012). Similar studies are lacking for the western stock and/or do not include comprehensive size, temporal, and/or spatial sampling. While age at maturity in the eastern stock has been established as 3–4 years (Rodríguez-Roda, 1967; Corriero et al., 2005), this parameter for the western spawning stock is the object of debate among fisheries managers. The International Commission for the Conservation of Atlantic Tunas (ICCAT) management paradigm assumes an age at 100% maturity for the western stock of 9 years (Anonymous, 2011), but historical studies indicate that some western bluefin tuna mature at age 4–6 years (Westman & Neville, 1942; Baglin, 1982). Goldstein et al. (2007) showed evidence of maturation in fish aged 7–8 years though this study was conducted on fishing grounds far from spawning areas, and individuals smaller than commercially legal size were not sampled. Recently, length data from Gulf of Mexico fisheries landings were used to estimate and increase the age at 50% maturity to 12–16 years (Diaz & Turner, 2007; Diaz, 2011). These studies made no direct observations of gonad tissues and estimated fish age from length.

Because eastern bluefin tuna presumably mature at a younger age (Rodríguez-Roda, 1967; Baglin, 1982; Medina et al., 2002; Corriero et al., 2005), they spawn for a greater proportion of their lifespan than western bluefin tuna (e.g., from age 3—20 years instead of 9–20 years). Consequently, the eastern stock is more productive than the western stock. However, the productivity and recruitment levels of both stocks still remain poorly understood (Fromentin & Powers, 2005), and large uncertainties remain for the western stock regarding reproductive output.

The lack of small/medium fish (< 185 cm, CFL and/or < 9 years old; NMFS management categories) sampled from the Gulf of Mexico spawning grounds suggests that Atlantic bluefin tuna segregate by size with smaller fish spawning in an alternative location (Bahamas, Caribbean, or Gulf Stream margins; (Mather et al., 1995; Lutcavage et al., 1999)). The mean body weight of eastern Mediterranean spawners was significantly lower than that of central and western Mediterranean spawners (Heinisch et al., 2008). Since small spawners (≈125 kg) were present in eastern, central, and western Mediterranean spawning locations, this could indicate a partial size segregation of bluefin tuna during the spawning season. Size segregation on spawning grounds has also been suggested for Pacific bluefin tuna (*Thunnus orientalis*; Itoh, 2006). Electronic tag data have shown many large, presumably mature, fish outside known spawning areas during the assumed spawning season (Lutcavage et al., 1999; Block et al., 2005; Galuardi et al., 2010) suggesting bluefin tuna may spawn elsewhere or do not spawn annually (Goldstein et al., 2007; Galuardi et al., 2010). The latter scenario seems unlikely for older, larger fish because skipped spawning is predicated to occur primarily in the first years of maturity (Chapman et al., 2011).

Electronic tagging and macroscopic examination of gonads are useful, but not sufficient for assessing population reproductive dynamics. Histological analysis of gonads allows accurate identification of maturation stages and, consequently, the character-

ization of the reproductive cycle (Medina et al., 2002). Bluefin gonad histology has been described extensively in the Mediterranean Sea (Rodríguez-Roda, 1967; Medina et al., 2002, 2007; Corriero et al., 2005). In the western and central Atlantic, gonad histology was examined in fish from the Bahamas and the mid-Atlantic Bight where a significant proportion of post-spawning females were sampled, but no ripe ovaries (containing hydrated oocytes), were found in the mid-Atlantic Bight (Rivas, 1954; Baglin, 1976, 1982). These studies provided limited information since they were not spatially or temporally comprehensive, and the samples were taken far from the spawning area. As a result, reproductive parameters of actively spawning fish, such as spawning frequency and/or fecundity, could not be determined (Clay, 1991).

Fecundity estimates allow the quantification of the reproductive capacity of individual fish and are essential for accurate assessment of the spawning stock (Murua et al., 2003). Assuming environmental characteristics between the Mediterranean Sea and Gulf of Mexico spawning grounds are different, and that Atlantic bluefin tuna exhibit natal homing, fecundity must be calculated separately for each stock as each stock may exhibit different fecundity. Realized and potential batch fecundities can be calculated by stereological counts of post-ovulatory follicles (POFs) and migratory-nucleus follicles (MNF), respectively (Aragón et al., 2010; Aranda et al., 2011).

In the present study, a histological and stereological comparison of gonads from bluefin tuna caught in the Gulf of Mexico and the Mediterranean Sea was undertaken to compare the reproductive parameters (spawning periodicity, spawning frequency, and fecundity) estimated between both known spawning grounds.

Methods

Sample collection

Spawning area	Month	Body mass conversion
GMX	April–May	$BM = 6.043 \cdot 10^{-5} SFL^{2.7794}$
	June	$BM = 4.404 \cdot 10^{-5} SFL^{2.837}$
MED	mid-June–mid-July	$BM = 1.9607 \cdot 10^{-5} SFL^{3.0092}$

Table 3.1. ICCAT conversion equations used to attain body weight (BW) of Atlantic bluefin tuna, *Thunnus thynnus*, for each month sampled in the Gulf of Mexico (GMX) and the Mediterranean Sea (MED).

Female *T. thynnus* were sampled from commercial fisheries in the Gulf of Mexico (GMX) and the Mediterranean Sea (MED) between 2007 and 2009 (Figure 3-1). National Marine Fisheries Service observers sampled fish from longline fishing vessels in the northern region of the Gulf of Mexico from February–July (n=147). Seven samples obtained in February (n=1) and March (n=6) were not included in the stereological analysis because of low monthly sample size. These fish were still included in the histological descriptions of gonad development. In the Mediterranean Sea, female bluefin tuna were sampled from longline fishing vessels on the western Mediterranean Sea spawning grounds from mid-June to mid-July 2008 (n=40). Either curved fork length (CFL; GMX) or straight fork length (SFL; MED) of each individual was measured to the nearest centimeter, and CFL was converted to SFL (Eqn 3.1). Body weight (BW) was calculated from SFL by location and timing of catch according to ICCAT conversions (Table 3.1). Ovaries were immediately removed and weighed onboard, and ovarian volume (OV) was calculated from ovarian weight (OW; Eqn. 3.2; Medina et al., 2007).

$$SFL = CFL \cdot 0.955 \tag{3.1}$$

$$OV = OW \cdot 0.9174 \tag{3.2}$$

Histology

A subsample ($0.5 - 1\ cm^3$) was removed from the central portion of one ovary and

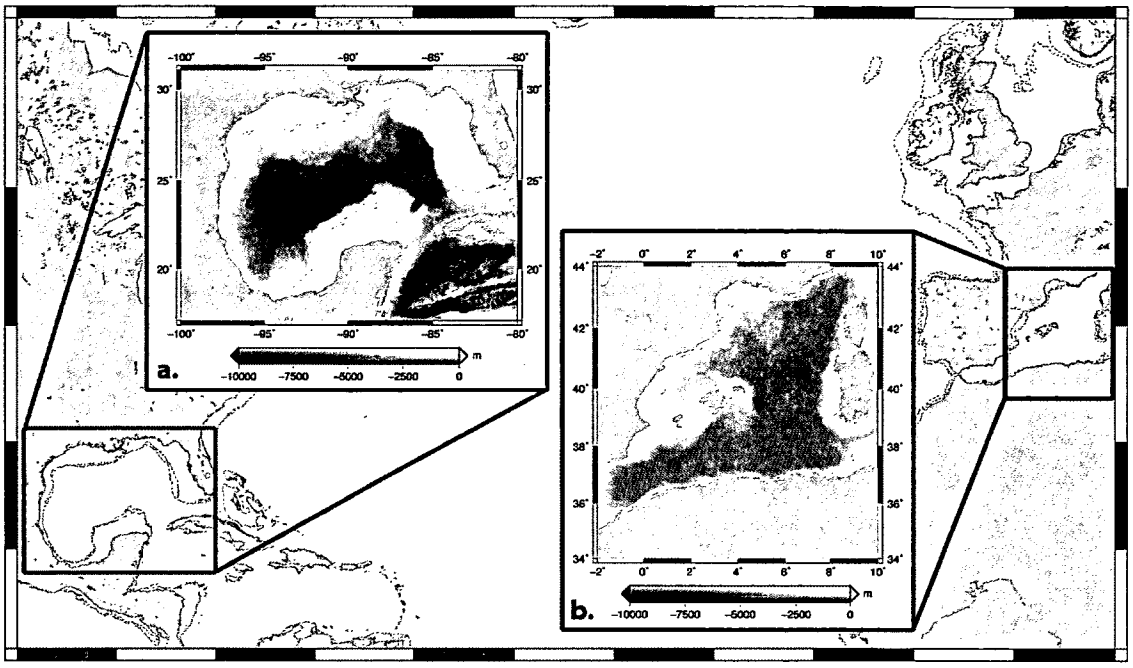

Figure 3-1. Map of the Gulf of Mexico (a) and Mediterranean Sea (b) sampling regions. Dotted lines represent the 100m and 200m depth contours, and the black and white border corresponds to latitude and longitude.

fixed for at least 24h in 4% formaldehyde (10% formalin) in phosphate buffer, 0.1 M, pH 7.2. Tissue samples were then dehydrated through increasing concentrations of ethyl alcohol, cleared with either xylene (MED) or ClearRite3® (GMX), and embedded in paraffin wax. Samples were cut into 5–6 μm sections using a microtome, stained with either haematoxylin-eosin (GMX) or haematoxylin-VOF (MED; Figure 3-2; Gutiérrez, 1967), and permanently mounted on slides using either Richard-Allan Scientific Mounting Medium (GMX; Thermo Scientific) or Eukitt (MED; Sigma Aldrich).

Five distinct types of developing follicles were distinguished depending on the respective oocyte developmental stages: perinucleolar (PNF), lipid-stage (LSF), vitellogenic (VF), and oocyte maturation (OMF), which consisted of migratory-nucleus (MNF) and hydrated (HF) follicles. Additionally, α- and β-stage atretic follicles

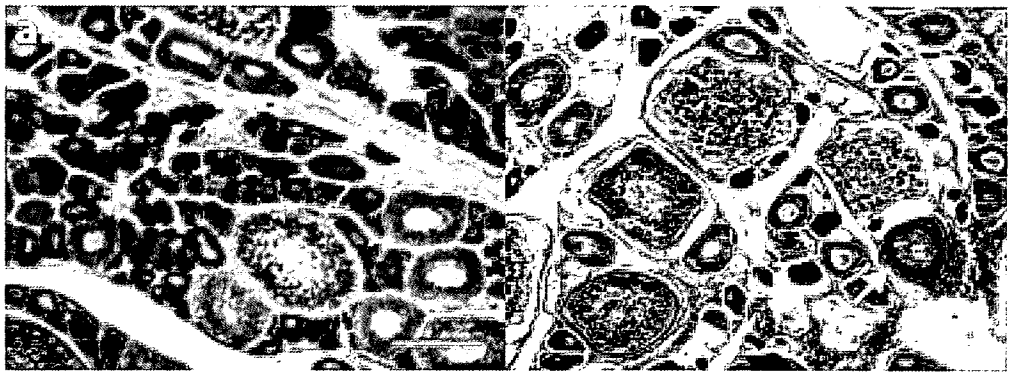

Figure 3-2. Comparison of the staining methods haematoxylin-eosin (a) used in the Gulf of Mexico and haematoxylin-VOF (b) used in the Mediterranean Sea. The orange color of the vitellogenic particles with the VOF stain make easy the identification of early vitellogenic oocytes (arrows), but this stain did not work with the Gulf of Mexico samples. Scale bars are 100 μm.

(αAF and βAF, respectively) and post-ovulatory follicles (POF) were counted (Figure 2-10). Based on the most advanced group of oocytes occurring in the ovary and the extent of atresia, fish were classified as inactive (IN), active non-spawning (ANS), or active spawning (AS; Schaefer, 1998, 2001).

Stereology

Stereological techniques can be used to estimate the proportion, and thereby the abundance, of different oocyte stages within any given ovary. The model-based stereology techniques applied in this study are a modified version of those described by Coward & Bromage (2002) according to Weibel & Gomez (1962) and previously used on bluefin tuna (Eqn. 3.3; Medina et al., 2002, 2007; Aragón et al., 2010),

$$N_V = \frac{K N_A^{1.5}}{\beta V_V^{0.5}} \tag{3.3}$$

where N_V is the numerical density of a given oocyte stage (number per unit volume).

N_A is the number of transections of oocytes per unit section area and was calculated as the number of oocytes within the stereological test system divided by the test area. Oocytes that border the upper and/or right margins of the digital images are counted while oocytes bordering the lower and/or left margins are rejected (Medina et al., 2002, 2007). V_V is the volume fraction occupied by oocytes in a given stage (herein: volume density) and was calculated by image analysis of ten digital micrographs for each ovary using ImageJ (Rasband, 1997–2011; Medina et al., 2007; Aragón et al., 2010). β is a shape coefficient (Eqn. 3.4), K is a size distribution coefficient (Eqn. 3.5); for complete derivation of both β and K, see Weibel & Gomez (1962),

$$\beta_{(\epsilon)} = \frac{4\pi}{3 \cdot \epsilon \cdot \Psi_{(\epsilon)}^{3/2}} \tag{3.4}$$

$$K = (\frac{D_3}{D_1})^{1.5} \tag{3.5}$$

where ϵ is a ratio of diameter over length, and for the (very lengthy) derivation of $\Psi_{(\epsilon)}$, see Weibel (1969). D_1 and D_3 are the first and third moment of the size distribution of oocytes (Weibel, 1969; Williams, 1977). All particles were considered ellipsoidal for the calculation of β according to Weibel (1969). Both β and K have been previously calculated for *T. thynnus*, and these values were used for this study (Table 3.2; Medina et al., 2002, 2007). The total number of follicles in each stage (N) was calculated by extrapolating N_V to the entire gonad volume (Eqn. 3.6). Gonad volume loss through processing was calculated (34.8% for MED and 43.3% for GMX), and correction factors were applied.

$$N = N_V \cdot GV \tag{3.6}$$

Follicle developmental stage	K	β
Lipid stage	1.07	1.43
Vitellogenic stage	1.03	1.42
Oocyte maturation stage	1.01	1.44
α-atresia	1.02	1.42
β-atresia	1.02	1.50
Post-ovulatory follicle	1.02	1.56

Table 3.2. Values of the coefficients K and β applied for the different follicle types (Medina et al., 2002, 2007). Oocyte maturation stage includes both migratory nucleus and hydrated stage oocytes.

For oocyte counting, as the section is designed to represent the entire gonad, there must be a homogeneous distribution of oocytes within the ovary for this method to be accurate. Homogeneous distribution of oocytes has been found for yellowfin tuna (June, 1953) and skipjack tuna (*Katsuwonus pelamis* (L.); Stéquert & Ramcharrun, 1995). A preliminary study of oocyte distribution in bluefin tuna sampled in the Gulf of Mexico showed homogeneous distribution of lipid and vitellogenic stage oocytes (Abdu, 2012) though the sample size was limited and other oocyte stages were not enumerated. A heterogeneous distribution of hydrated oocytes is likely since hydration begins at the periphery and spreads inward (Hunter et al., 1985). Several tuna species have been reported to have an uneven distribution of oocytes within one section between the periphery and central regions (Rivas, 1955; Otsu & Uchida, 1959; Baglin, 1982) so a heterogeneous distribution within sections cannot be ruled out.

Spawning frequency

Spawning frequency was estimated using the post-ovulatory follicle method (Eqn. 3.7; Stauffer & Picquelle, 1981) as adapted by Hunter & Macewicz (1985). This method calculates the mean spawning fraction as the total number of spawning females whose ovaries show postovulatory follicles (POFs) divided by the total number of mature

females sampled and corrects for biases in the numbers of females with hydrated oocytes.

$$F = \frac{M_{li}}{2M_{li} + m_{ni}} \tag{3.7}$$

where, F = fraction of females spawning per day, M_{li} = number of females with 1-day old post-ovulatory follicles (POFs), and m_{ni} = the number of females with no spawning history (females with POFs and/or hydrated or migratory nucleus oocytes are excluded). For bluefin tuna, POFs are reabsorbed within 24h so F is the proportion of females spawning within a 24h period.

Statistics

Comparisons of means of the stereological and biometrical parameters among years were performed using the Kruskal-Wallis test, and parameters with no significant difference were regrouped by month ($\alpha = 0.05$). Monthly variation was also analyzed using the Kruskal-Wallis test ($\alpha = 0.05$). The Mann-Whitney U-test with Bonferroni correction was used to assess significant differences between pairs of months ($\alpha = 0.0125$; Sokal & Rohlf, 1998; Zar, 1999).

Results

Female bluefin tuna were sampled from the Gulf of Mexico in 2007–2009 (n = 147, 172–326 cm, SFL), and from the Mediterranean Sea in 2008 (n = 45, 120–240 cm, SFL). The GSI was 0.32–6.9 in the Gulf of Mexico, and 0.30–5.8 in the Mediterranean Sea. Mean fork length (SFL) of bluefin tuna sampled in the Gulf of Mexico (235.61 ± 19.81) was significantly larger than that for fish sampled in the Mediterranean Sea (199.19 ± 27.21; Mann-Whitney, $p < 0.001$). No significant differences in SFL were

observed within the Gulf of Mexico throughout the sampling period (Kruskal-Wallis, $p > 0.05$).

The mean GSI was significantly higher in fish sampled from the the Mediterranean Sea than in fish sampled in June from the Gulf of Mexico (Kruskal-Wallis, $p = 0.0295$), and there was no significant difference in GSI within the Mediterranean Sea during the sampling period (Tukey-Kramer HSD, $p > 0.05$). Within the Gulf of Mexico, there was no difference in the GSI across the sampling period (Table 3.5).

Histology

Results of histological analyses of females from the Gulf of Mexico presented here are based on a slightly different classification scheme than in Chapter 2 to allow direct comparison with the Mediterranean samples. Here, fish were classified as active non-spawning (ANS; Figure 3-3) rather than inactive-mature stage (Figure 2-9 c,d). The three classifications used in this chapter are active spawning (AS), active non-spawning (ANS), and inactive (IN).

Histological analysis of ovarian tissue from the Gulf of Mexico sampled from April to June showed no significant differences in gonad development between years. The number of inactive females was less than 20% of our sample except in 2007 (28.0%). The proportion of active non-spawning (ANS) females was 20.0% (2007), 50.0% (2008) and 30.0% (2009). The proportion of active spawning (AS) females was consistent at about 50% for all years (Figure 3-4). Given the lack of annual variation, Gulf of Mexico samples were pooled for all years for subsequent analyses.

When ovary samples were arranged by month, consistent differences in the reproductive condition were observed with increasing maturation throughout the sampling period. In the Gulf of Mexico, samples collected in February and March (n=8) did not include any AS females. The proportion of AS females increased from April to

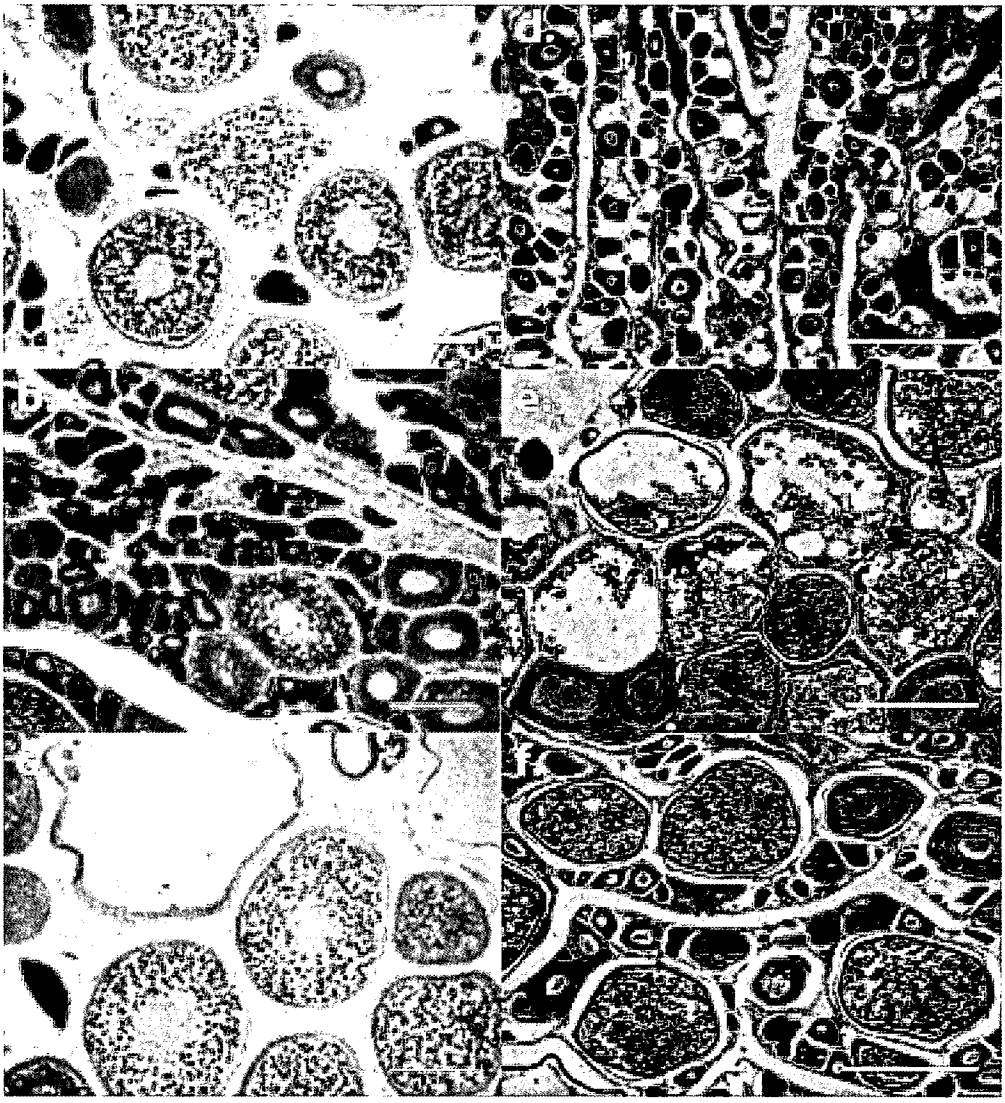

Figure 3-3. Maturity stages assigned to Atlantic bluefin tuna, *Thunnus thynnus*: (a–c) Gulf of Mexico; (d–f) Mediterranean Sea; (a,d) inactive (IN); (b,e) active non-spawning (ANS); (c,f) active spawning (AS). Scale bars are 250 μm.

June until a small decline was observed near the end of June. Near the end of the presumed spawning season in the Mediterranean Sea (mid-June–mid-July), proportions of AS, ANS, and IN fish were similar to those observed in the Gulf of Mexico in May (Figure 3-5).

Spawning frequency

The spawning fraction in the Mediterranean Sea samples was higher than in the Gulf of Mexico. Spawning fraction in the Gulf of Mexico was estimated to be 0.45 throughout the sampling period where 49 out of 108 mature females contained POFs in their ovaries. When this parameter was calculated by month, the proportion of females with POFs sampled in April was lower than that in May and June. When the spawning frequency was calculated considering only AS females, its value increased significantly and remained similar among months. All Mediterranean Sea AS females had POFs, and the spawning fraction was estimated to be 0.60. (Table 3.4).

Stereology

Stereological counts of the different stages of oocyte development revealed significant differences between the Mediterranean Sea and the Gulf of Mexico with regard to LSF, VF, and βAF (Table 3.5). Stereological counts of α-atretic follicles (αAF and βAF) did not change significantly in the Gulf of Mexico throughout the sampling period. The numerical density of βAF ($N_V\beta$AF; number per mm^3) and the relative number of βAF ($Ng^{-1}\beta$AF; number per g) observed in eastern females were significantly higher than in the Gulf of Mexico for several months (Kruskal-Wallis, $p < 0.0125$).

No significant differences were found in the numerical density of LSF (N_VLSF) within the Gulf of Mexico throughout the sampling period. Nevertheless, in the Gulf of Mexico the total number of LSF (NLSF) was significantly higher in April, and

the relative number of LSF (Ng^{-1}LSF) was significantly higher in April and May. In general, stereological counts of LSF quantified in the Mediterranean Sea were significantly lower than those from the Gulf of Mexico sampled in April and May.

The numeric density of VF (N_VVF) in the Gulf of Mexico was significantly lower in April than in May (Tukey-Kramer HSD, $p = 0.0222$) but the mean number of VF (NVF) remained unchanged in the Gulf of Mexico throughout the sampling period. The relative number of VF (Ng^{-1}VF) was significantly higher in May than in June (Kruskal-Wallis, $p = 0.0383$), but there was no significant difference relative to April. N_VVF in the Mediterranean Sea was statistically similar to Gulf of Mexico values in May and June, and NVF estimated in the Mediterranean Sea were similar to the Gulf of Mexico values in all months. However, because eastern fish were generally smaller than western fish on average, the relative number of VF (Ng^{-1}VF) was much higher for Mediterranean Sea fish (Table 3.5).

The low number of females with MNF and HF (n=11 and n=4, respectively) is likely the cause for finding no significant differences for these stages throughout the sampling period in the Gulf of Mexico. For the Gulf of Mexico fish, the highest number of POFs (N_VPOF, NPOF and Ng^{-1}POF) occurred at the beginning of the sampling period (April) and the lowest values were observed in June; however, there was no significant difference either among months. Additionally, there was no significant difference for any POF values between the spawning areas. The total mean number of POFs (±SD) estimated for fish sampled in the Gulf of Mexico was $7.65 \cdot 10^6(\pm 6.71 \cdot 10^6)$, which corresponds to a relative batch fecundity (Ng^{-1}) of 28.14(±26.90) POF g^{-1} while the total mean number of POFs (±SD) estimated for fish sampled in the Mediterranean Sea was $7.36 \cdot 10^6(\pm 6.71 \cdot 10^6)$ corresponding to a relative batch fecundity (Ng^{-1}) of 45.56(±33.79) POF g^{-1}.

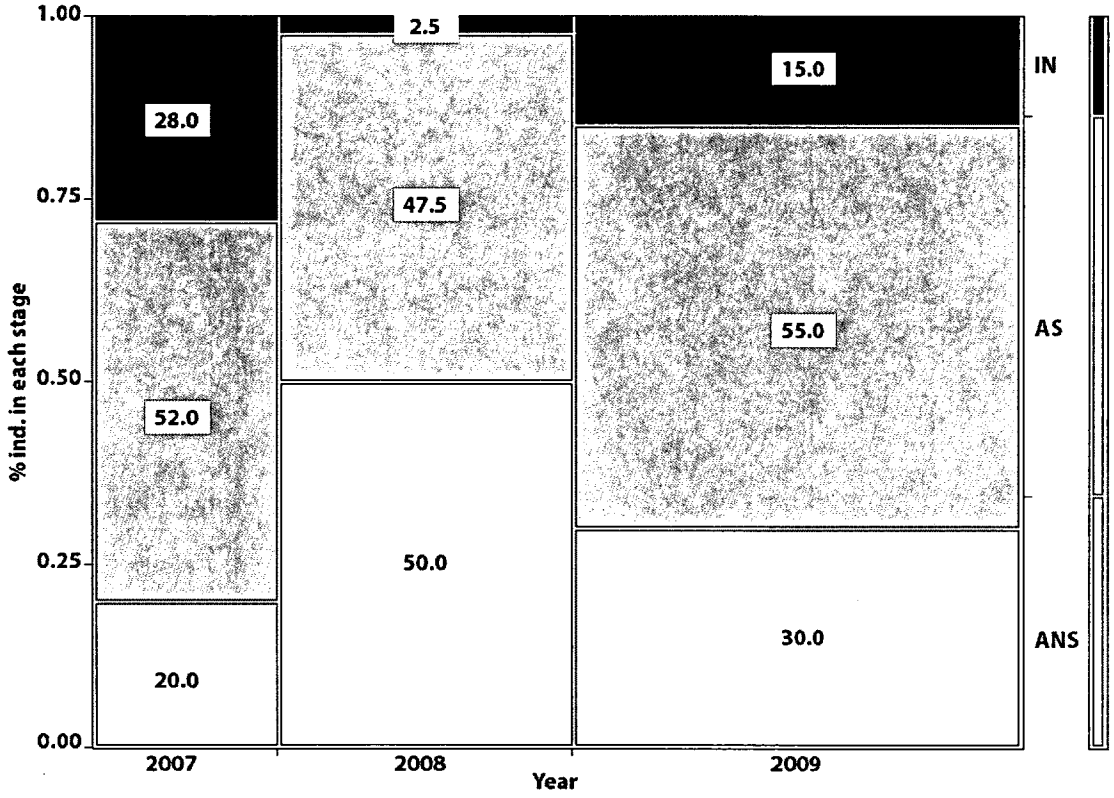

Figure 3-4. Percent of Atlantic bluefin tuna, *Thunnus thynnus*, sampled from the Gulf of Mexico classified in each stage of reproductive maturity by year. No statistical difference was found among years for each stage. Column widths are representative of sample size (2007 n=26; 2008 n=46; 2009 n=60)IN=inactive; ANS=active non-spawning; AS=active spawning.

Spawning area	Year	Month	n	Straight fork length (cm)			GSI (%)
				Mean±SD	min.	max.	Mean±SD
GMX	2007	April–June	26	235.66 ± 21.09	172	262	2.72 ± 1.61
	2008	April–June	47	237.31 ± 20.40	184	326	2.76 ± 1.07
	2009	April–June	74	234.72 ± 19.09	191	285	2.46 ± 0.99
MED	2008	mid-June–mid-July	45	199.19 ± 27.21	120	240	3.05 ± 1.45

Table 3.3. Biometric data from Atlantic bluefin tuna, *Thunnus thynnus*, caught on longline fishing vessels operating in the Gulf of Mexico (GMX) and Mediterranean Sea (MED). SFL=straight fork length; GSI=gonadosomatic index.

Spawning area	Month	Total	AS	With POFs	Spawning frequency		Spawning interval
					(Total)	(AS only)	(days)
GMX	April	30	12	9	0.30	0.75	3.33
	May	68	45	35	0.51	0.78	1.94
	June	10	7	5	0.50	0.71	2.00
	all months	108	64	49	0.45	0.77	2.20
MED	June/July	40	24	24	0.60	1.00	1.67

Table 3.4. Spawning frequency and spawning interval estimates for Atlantic bluefin tuna, *Thunnus thynnus*, sampled from the Gulf of Mexico (GMX) and the Mediterranean Sea (MED). AS=active spawning; POF=post-ovulatory follicle.

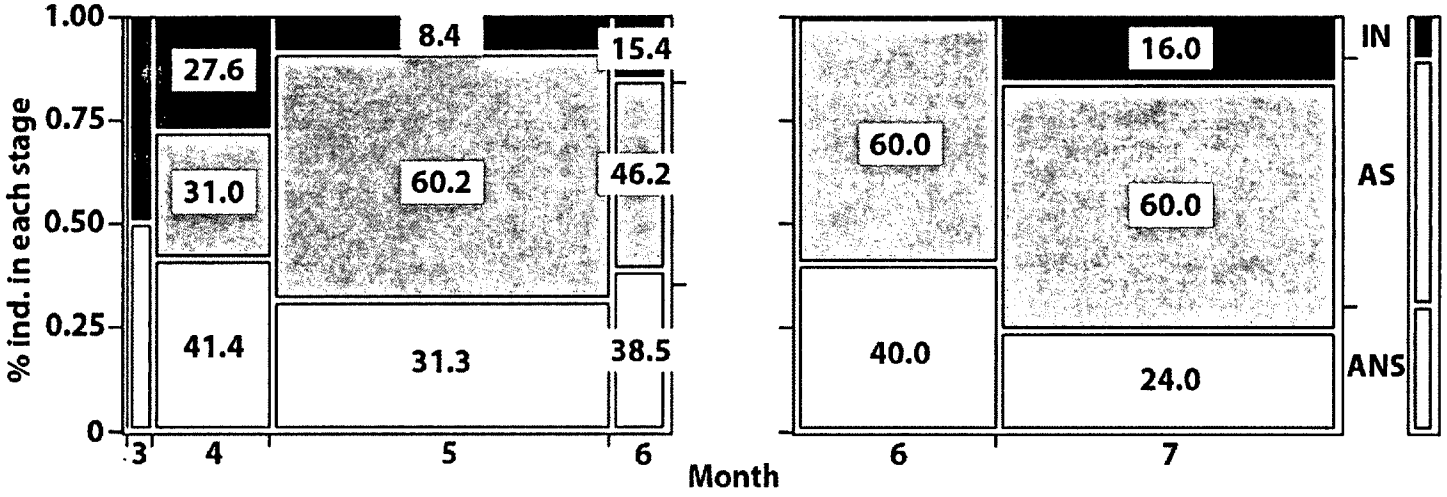

Figure 3-5. Percent of Atlantic bluefin tuna, *Thunnus thynnus*, sampled from the Mediterranean Sea (MED; right) and the Gulf of Mexico (GMX; left) classified in each stage of reproductive maturity by month. The six samples collected in March were 50% IN and 50% ANS. The one fish sampled in February was IN and is not shown in the figure. Column widths are representative of sample size (GMX: March n= 6; April n=29 May n=83; June n=13; MED: June n=15; July n=25)IN=inactive; ANS=active non-spawning; AS=active spawning.

		GMX								MED		
		April			May			June		June/July		
		n	Mean±SD		n	Mean±SD		n	Mean±SD		n	Mean±SD	
(cm)	SFL	32	241.35 ± 25.27	a	100	233.08 ± 17.80	a	15	241.30 ± 16.28	a	42	198.12 ± 27.61	b
	GSI	27	2.76 ± 1.20	a,b	68	2.67 ± 1.09	a,b	12	1.75 ± 0.76	b	39	3.05 ± 1.45	a
$\frac{Nv}{mm^3}$	αAF	26	0.997 ± 0.54		45	1.02 ± 0.771		6	0.937 ± 0.607		29	1.74 ± 2.22	
	βAF	19	0.957 ± 0.70	a	29	0.761 ± 0.53	a	4	1.02 ± 1.02	a,b	35	2.72 ± 3.39	b
	LSF	29	23.40 ± 7.28	a	82	19.25 ± 7.97	a,b	13	18.22 ± 10.03	a,b	41	17.58 ± 10.82	b
	VF	29	5.05 ± 1.74	a	83	6.00 ± 1.79	b	13	5.76 ± 2.37	a,b	36	6.29 ± 2.18	b
	MNF	0			9	0.47 ± 0.29		2	0.41 ± 0.50		4	0.48 ± 0.34	
	HF	1	0.59		3	0.37 ± 0.38		0			0		
	POF	9	1.40 ± 1.09		45	1.22 ± 0.93		5	1.06 ± 0.54		24	1.46 ± 0.94	
$N(\times 10^6)$	αAF	22	7.64 ± 5.75		31	6.72 ± 5.15		4	7.31 ± 7.89		27	7.19 ± 8.91	
	βAF	16	7.31 ± 7.04		21	5.57 ± 4.84		3	11.20 ± 16.27		33	8.58 ± 7.39	
	LSF	25	165.25 ± 84.79	a	59	122.41 ± 63.03	b	10	79.04 ± 59.56	b,c	39	77.15 ± 60.29	c
	VF	25	34.18 ± 19.11		60	38.87 ± 18.55		10	25.61 ± 9.36		34	32.44 ± 19.19	
	MNF	0			7	3.20 ± 2.03		2	2.01 ± 2.55		4	2.34 ± 1.67	
	HF	1	4.89		2	0.829 ± 0.108		0			0		
	POF	8	10.25 ± 9.54		34	7.57 ± 6.24		5	4.29 ± 3.01		23	7.36 ± 6.17	
$\frac{N}{g}$	αAF	22	27.04 ± 20.01		30	25.92 ± 15.97		4	22.05 ± 19.03		27	41.49 ± 46.58	
	βAF	16	28.79 ± 32.91	a,b	21	19.56 ± 16.78	a	3	30.38 ± 41.74	a,b	33	58.31 ± 54.68	b
	LSF	25	579.15 ± 248.21	a	58	455.30 ± 216.89	a,b	10	282.62 ± 214.98	b	39	462.75 ± 318.73	b
	VF	25	120.90 ± 61.39	a	59	144.59 ± 59.77	a,b	10	91.92 ± 26.75	a,c	34	200.64 ± 99.33	d
	MNF	0			7	11.91 ± 5.67		2	7.64 ± 9.86		4	14.61 ± 8.13	
	HF	1	23.63		2	3.00 ± 0.53		0			0		
	POF	8	40.42 ± 45.62		33	27.20 ± 22.10		5	14.73 ± 7.58		23	45.56 ± 33.79	

Table 3.5. Stereological data for Atlantic bluefin tuna, *Thunnus thynnus*, sampled in the Gulf of Mexico (GMX) and Mediterranean Sea (MED). Different letters in columns indicate significance. SFL=straight fork length; GSI=gonadosomatic index; αAF=alpha atretic; βAF=beta atretic; LSF=lipid stage; VF=vitellogenic; MNF=migratory nucleus; HF=hydrated; POF=post-ovulatory follicles; $\frac{N_v}{mm^3}$=numerical density; $N(\times 10^6)$=total number; $\frac{N}{g}$=relative density.

Discussion

Although not spatially and temporally exhaustive, this study represents the first attempt since the 1970's to assess the spawning condition of Atlantic bluefin tuna sampled on the Gulf of Mexico spawning grounds and is the first histological comparison of the eastern and western spawning stocks.

Histological analysis of gonad samples from the spawning grounds throughout the spawning season is essential for evaluating the reproductive condition and performance (fecundity, spawning periodicity, etc.) of bluefin tuna. Additionally, systematic sampling on the spawning grounds can identify temporal variations in key reproductive parameters such as sex ratio, proportion of mature fish, spawning frequency, and spawning periodicity. This information was lacking for the western stock resulting in large uncertainties for stock assessment and evaluation of productivity (Fromentin & Powers, 2005). Since the implementation of the moratorium on directed fishing in the Gulf of Mexico, federal fisheries observers have sampled bluefin tuna caught as bycatch in the yellowfin tuna and swordfish longline fisheries (*Thunnus albacares* and *Xiphius gladius*, respectively). Such restrictions prohibit comprehensive spatial, temporal, and size sampling of spawning bluefin tuna in the Gulf of Mexico. In this study, the smallest bluefin tuna sampled from the Gulf of Mexico, 172 cm (SFL) and estimated age 7–8 years (Restrepo et al., 2010), had ripe ovaries with numerous recent POFs. This does not support the current assumption of maturity at 9 years (Anonymous, 2011) or the increase recently proposed from landings length data of 12–16 years (Diaz & Turner, 2007; Diaz, 2011). In order to fully understand the reproductive dynamics of the western spawning stock, the maturity ogive should be revised using comprehensive size sampling over larger temporal scales including histological examination of the ovaries and endocrine profiling (Rosenfeld et al., 2003, 2012).

As a consequence of determining the maturity ogive of western bluefin tuna based on longline bycatch sampling in the Gulf of Mexico, previous studies have lacked small/medium fish (<180 cm) leading to biased results of larger/older size and age at maturity estimates (Diaz & Turner, 2007; Diaz, 2011). Additionally, these studies assumed bluefin tuna only spawn in the Gulf of Mexico despite the lack of spatially comprehensive sampling and historical reports of bluefin spawning outside the Gulf of Mexico (Rivas, 1954; Mather, 1962; Baglin, 1976; Mather et al., 1995). Goldstein et al. (2007) suggested previous bluefin tuna sampling has not accurately represented the spawning size range of the western population because it only included fish sampled by longline fishing vessels operating on known spawning grounds rather than all size classes sampled throughout their range. Gear type, size selectivity, and vertical distribution of tuna by size also influence the size of spawners sampled by commercial fishing fleets (Davis & Farley, 2001; Medina et al., 2007). Since yellowfin tuna caught by longline fishing vessels are, on average, 120 cm (Schaefer, 1998), it would be expected that similar sized bluefin tuna would be captured. However, prior to the US moratorium, catch records indicated the presence of only giant bluefin tuna (>180 cm) in the Gulf of Mexico (Mather et al., 1995). As opposed to the current management paradigm of western bluefin tuna maturing at an older age than the eastern stock, fish may exhibit size and temporal segregation on the spawning grounds as is seen with the eastern spawning stock (Mather et al., 1995; Lutcavage et al., 1999; Heinisch et al., 2008) and in Pacific bluefin tuna (*Thunnus orientalis*; Itoh, 2006). There is indirect evidence that smaller fish utilize alternative spawning locations, such as the Caribbean Sea, the Bahamas, or the Gulf Stream margins because ripe fish have been sampled there (Rivas, 1954; Wilson & Bartlett, 1967; Mather et al., 1995). Given this evidence, it is possible that smaller bluefin tuna spawn in alternative locations within the Gulf of Mexico as previous sampling has been spatially concentrated in

the north/central Gulf where US longline vessels operate and temporally restricted to March–July. A bluefin tuna life history model predicts that smaller/younger maturing migratory fish should have shorter migration routes and spawn in areas closer to feeding areas than larger, older fish with higher energy reserves(Jørgensen & Fiksen, 2006; Jørgensen et al., 2008; Chapman et al., 2011).

Electronic tagging results have consistently shown annual migration patterns of giant bluefin tuna not entering either known spawning ground before returning to northern feeding grounds (Lutcavage et al., 1999; Block et al., 2005; Sibert et al., 2006; Galuardi et al., 2010). It is possible that western bluefin tuna spawn over a broader area of regions with oceanographic conditions appropriate for larval development than previously assumed (Mather et al., 1995; Lutcavage et al., 1999; Goldstein et al., 2007; Galuardi et al., 2010). Recent larval cruises found bluefin tuna larvae outside the Gulf of Mexico (Muhling et al., 2011a), but spawning areas beyond the northern Gulf of Mexico await histological validation. Pop-up satellite tagged 2–5 year old bluefin tuna did not enter the Gulf of Mexico or Mediterranean Sea during presumed spawning periods (April–June). Nevertheless, some fish lingered areas visited by tagged adults (Block et al., 2001, 2005; Wilson et al., 2005; Sibert et al., 2006) in subtropical seas north of the Bahamas and in the southern mid-Atlantic Bight and other regions (Galuardi & Lutcavage, 2012).

Our histological examination of the Gulf of Mexico bluefin tuna ovaries revealed differences in follicle maturation between months throughout the spawning period. While samples collected in February and March contained no active spawning (AS) individuals, 31% of samples collected in April were AS individuals. As the spawning season progressed, the number of AS females increased and peaked in May (60%). While not statistically significant, the GSI observed in the Gulf of Mexico fish decreased throughout the sampling period indicating a decrease in ovarian size, and

thus, the cessation of the spawning season. The proportion of AS females from the Mediterranean Sea in June/July was most similar to Gulf of Mexico females sampled in May indicating peak spawning activity in the northern Gulf of Mexico is slightly earlier than in the western Mediterranean Sea.

The statistical results of the stereological analysis are consistent with previous findings and with the progression of the spawning season (Medina et al., 2002). As the spawning season progresses, LSF become less frequent indicating high levels of recruitment to VF thereby compensating for losses caused by atresia or spawning. Similarly, the relative number of VF (Ng^{-1}VF) was significantly higher in May than in June indicating a decrease in the recruitment of VFs as the end of the spawning season approaches.

In spawning fish, atresia is a natural mechanism for regulating the number of eggs spawned (Kurita et al., 2003; Kraus et al., 2008; Skjæraasen et al., 2010). Alternatively, massive atresia can indicate a cessation of oocyte maturation and/or spawning activity (Rideout et al., 2000). The Gulf of Mexico samples showed relatively low and stable levels of αAF throughout the spawning season indicating bluefin tuna sampled in the Gulf of Mexico were in favorable condition for oocyte maturation and spawning. While these fish appeared to be actively spawning, tagged bluefin tuna of presumed reproductively mature size observed outside the known spawning areas during the spawning period could be skipping spawning due to unfavorable body condition (Rideout et al., 2000, 2005; Rideout & Rose, 2006; Goldstein et al., 2007; Galuardi et al., 2010). Bluefin tuna sampled on the New England and Canadian foraging grounds have had periods of reduced somatic condition (Golet et al., 2007; Paul et al., 2011) possibly accounting for an increased incidence of skipped spawning (Rideout et al., 2005; Rideout & Tomkiewicz, 2011). However, the incidence of skipped spawning in bluefin tuna is unknown, and modeling results show it is less

likely to occur in larger, older fish in positive energy balance (Chapman et al., 2011). Giant bluefin tuna sampled on western foraging grounds in the fall (Goldstein et al., 2007; Golet et al., 2007) and in the Mediterranean Sea in the spring and early summer (Mourente et al., 2002) have extensive perigonadal fat and somatic lipid stores, and thus seem unlikely candidates for skipped spawning (Lutcavage et al., 2012).

While bluefin tuna have been observed on the western spawning grounds as early as November (Galuardi et al., 2010), individuals are believed to be actively spawning for only a few weeks (Richards, 1976; Baglin, 1982). These findings, albeit fishery-dependent, define the temporal borders of the reproductive events occurring in the north/central part of the Gulf of Mexico indicating the spawning season there spans April through June with maximum spawning activity in May. However, bluefin tuna begin entering the Gulf of Mexico in late November, and those arriving in winter experience warm water masses of $\geq 24°C$ in some areas of the Gulf of Mexico (Galuardi et al., 2010). Reproductive sampling has been primarily conducted in the northern Gulf of Mexico and US territorial seas in late spring (Richards, 1976; Baglin, 1982). Spawning activity occurring earlier in other areas of the Gulf of Mexico awaits confirmation by broader biological sampling, especially in Mexican territorial seas as historical studies report bluefin tuna larvae in these areas (Montolio & Juarez, 1977).

The relatively low proportion of Gulf of Mexico females with POFs in their ovaries (<51%) contrasts with the high spawning frequency (60%) observed in the western Mediterranean Sea (Medina et al., 2007; Aranda et al., 2012). The lower spawning frequency observed in the Gulf of Mexico could be the result of bias associated with utilizing the yellowfin and swordfish fisheries as the only sampling method (Medina et al., 2007). As long as bluefin tuna reproductive studies rely on bycatch in commercial fisheries, it is not possible to obtain an unbiased, accurate assessment of bluefin tuna reproduction. Given these constraints, it is important to note the temporal and

spatial aspects of the sampling as well as the fishing gear used for any bluefin tuna maturity study.

Stereological methods have often been used as an accurate tool for estimating fecundity in fishes, including eastern bluefin tuna (Coward & Bromage, 2002; Medina et al., 2002; Murua et al., 2003; Aragón et al., 2010; Kjesbu et al., 2011). Realized fecundity can be estimated through stereological counts of POFs, whereas the number of MNF is an estimation of the potential fecundity (Aragón et al., 2010; Aranda et al., 2011). In this study, the mean relative batch fecundity was calculated directly from stereological counts of POFs and showed a decrease as the season progressed. This is atypical for indeterminate spawners (Murua et al., 1998, 2006) but is likely due to the selective nature of sampling bluefin tuna as bycatch in a longline fishery (Murua et al., 1998; Murua & Motos, 2006). Kjesbu et al. (1998) suggested that monthly variation of fecundity may be masked by a decrease in the condition factor of fish appearing later on the spawning ground. Although significant differences were not found between months for our samples, the highest value in the relative fecundity occurred early in the season (April), even though the number of AS females was still quite low. Bluefin tuna entering the Gulf of Mexico early might exhibit higher reproductive potential than those arriving later due to the good condition acquired on the foraging grounds (Lutcavage et al., 2012). Additionally, bluefin tuna entering the Gulf of Mexico early (November, December, January) may be continuing to forage prior to the onset of the spawning season. Otherwise, the lower spawning frequency observed on the western spawning grounds could be a consequence of migration distance (Chapman et al., 2011) or decreased body condition observed on the western foraging grounds (Golet et al., 2007). While being the first to arrive on the spawning grounds might provide increased resource availability for offspring, arriving in poor condition could decrease larval survival rates (Mourente et al., 2002; Donelson et al., 2009).

The fecundity of eastern spawning bluefin tuna was estimated at 59 eggs g^{-1} (Medina et al., 2007) and 48.22 eggs g^{-1} (Aranda et al., 2012) for potential and realized fecundity, respectively. This realized fecundity is similar to the 45.56 eggs g^{-1} observed in this study for Mediterranean Sea spawners. The realized fecundity estimated for Gulf of Mexico spawners in this study was 28.14 eggs g^{-1}, though there was no significant difference in fecundity between the two spawning areas. Aranda et al. (2012) showed that realized fecundity is not proportional to body size, and thus, we would not expect to see a difference between the two stocks despite the large difference in body size. However, Witthames et al. (1995) showed that within a given species, fecundity could vary as a result of different adaptations to environmental habitats. It is possible that the difference in the fecundity estimated from fish from the two spawning areas is a result of the protracted temporal and spatial sampling in the Gulf of Mexico. Further sampling of actively spawning fish on known and potential western spawning grounds is warranted to clarify the issue of fecundity in the two spawning stocks.

Eastern and western bluefin tuna spawning sites seem to exhibit the same periodicity (three months), but spawning in the northern Gulf of Mexico occurs one month earlier than in the western Mediterranean spawning ground though sampling in the Gulf of Mexico is spatially and temporally limited. The earlier start to the western spawning is possibly due to specific oceanographic conditions and the early warmer temperatures observed in the Gulf of Mexico (García et al., 2003; Lohrenz & Verity, 2004). In this study, we have observed similar values in bluefin tuna reproductive parameters showing that the spawning condition of Mediterranean spawners from mid-June to mid-July is comparable with the reproductive peak observed in the Gulf of Mexico in May.

A more holistic view of the population dynamics of Atlantic bluefin tuna requires that its life history characteristics, reproductive profiles, and spawning areas and periodicity are well defined, especially since they undoubtedly will change with shifts in climate and ocean productivity (Cushing, 1982; Roessig et al., 2004; Lehodey et al., 2006; Caballero-Alfonso et al., 2009; Muhling et al., 2011b). The extent and quality of lipids acquired by tunas before they arrive in spawning areas will affect eggs and larvae (Mourente et al., 2002; Chapman et al., 2011). Future work should address energetic relationships between reproduction, migration, and early life history through modeling, biological sampling, and the development of smart tags to detect spawning.

CHAPTER 4

REPRODUCTIVE CONDITION OF ATLANTIC BLUEFIN TUNA SAMPLED FROM THE NORTHWEST ATLANTIC FORAGING GROUNDS

Introduction

The reproductive biology of Atlantic bluefin tuna, *Thunnus thynnus*, remains poorly understood and riddled with inconsistencies despite the high economic value of this fishery and its highly depleted status. While fish sampled directly from the spawning grounds are crucial for answering questions about fecundity, spawning intervals, etc., the sampling conducted in the Gulf of Mexico (GMX) is highly selective, biased towards large fish, and does not allow for comprehensive size sampling. Sampling fish from the foraging grounds allows a wider range in size sampling and better represents the entire stock (Fromentin & Powers, 2005). However, as bluefin tuna have a high metabolic rate and reabsorb signs of maturity quickly (post-ovulatory follicles, vitellogenic oocytes, etc.), assessing maturity in foraging fish can be challenging (Goldstein et al., 2007) and requires the use of indirect methods. The issue of reproductive maturity is not resolved for Atlantic bluefin tuna yet is key for determining the feasibility of a successful stock recovery (Mather et al., 1995; Fromentin & Powers, 2005; Jørgensen et al., 2006).

Historic studies suggested western bluefin tuna mature between the ages of 5–7 years (Westman & Neville, 1942; Wilson, 1965; Baglin, 1982; Locke, 1995; Mather et al., 1995), with some fish maturing at age 4 (Westman & Neville, 1942; Mather et al., 1995). In contrast, individuals sampled in the Gulf of Mexico, a known spawn-

ing ground, were considerably larger/older (8–12 years; Richards, 1976; Baglin, 1982). Recent studies proposing an increase in the age at maturity to 12–16 years (Diaz & Turner, 2007; Diaz, 2011) did not examine gonad tissues to confirm maturity. Additionally, these studies relied entirely on estimated ages from fish length from bluefin tuna collected as bycatch from the yellowfin and swordfish longline fisheries operating in the north/central region of the GMX in the spring. Despite these discrepancies in age at maturity, 9 years is the age currently assumed for western bluefin tuna sexual maturation (Anonymous, 2011). This is much older than the age at maturity of 3–4 years for eastern bluefin tuna (Tiews, 1963; Rodríguez-Roda, 1967; Susca et al., 2001a; Karakulak et al., 2004b; Corriero et al., 2005) despite the recent determination of similar growth curves for the two stocks (Restrepo et al., 2010). Since reproduction is a major inhibitor of growth in teleosts (Evans & Claiborne, 2006; Barton, 2007), western bluefin tuna should exhibit faster growth rates than the presumed earlier maturing eastern bluefin tuna. Stable isotope analyses and foraging studies have shown that juveniles from each margin of the Atlantic basin share trophic position and ecological traits (Logan et al., 2011) and mix extensively on the NW Atlantic shelf (Rooker et al., 2008a; Dickhut et al., 2009).

Since 2000, Large Pelagics Research Center (LPRC) scientists have conducted biological sampling of fish landed by the commercial fishery on the Gulf of Maine and southwest Nova Scotia foraging grounds during summer and fall to determine reproduction and maturity status of western bluefin tuna. In 2008, we began more extensive sampling to include smaller fish caught by the recreational fishery to help get a more accurate assessment of reproductive maturity in western bluefin tuna.

Distinguishing resting, non-reproductive ovaries from immature ovaries can be difficult, even with histology, because they contain the same oocyte size-frequency distribution as immature ovaries (Goldstein et al., 2007; Schirripa, 2011). Burton &

Idler (1984) reported that the ovarian wall thickness of winter flounder, *Pseudopleuronectes americanus*, increased post-spawning and continued to appear thicker than immature flounder outside of the spawning season. This has also been reported for wahoo, *Acanthocybium solandri*, and proved to be useful for identifying mature, regressed fish (Maki Jenkins & McBride, 2009). Additionally, the post-ovulatory follicle (POF) is formed when the oocytes are ovulated and serve as an unmistakable indicator of previous spawning. However, POFs are highly seasonal and can degrade shortly within a few hours after the spawning event (Clarke, 1987; Hunter & Goldberg, 1980; Isaac-Nahum et al., 1988).

Despite these challenges, bluefin tuna sampled on the foraging grounds still contain signs of maturity and can provide useful information for the bluefin tuna maturity ogive (Goldstein et al., 2007). The objectives of this study are to assess the maturity status of male and female Atlantic bluefin tuna sampled from the northwest Atlantic foraging grounds between Nantucket, Massachusetts, USA and Nova Scotia, Canada. Rather than focusing solely on the spawning stock, sampling the foraging grounds provides a more comprehensive size representation of the entire stock.

Methods

Sample Collection

From 2004–2011, samples of male and female bluefin tuna gonads were collected from commercial and recreational fisheries landings from the the Gulf of Maine and southwest Nova Scotia (NW Atlantic), including but not limited to the Nantucket Shoals, Jeffrey's Ledge, Georges Bank, and Stellwagen Bank (Figure 4-1). These specific locations are highly productive forage grounds occupied by bluefin tuna from May through October (Crane, 1936; Mather et al., 1995; Wilson et al., 2005).

Curved fork length (CFL) was measured to the nearest centimeter. For body weight (BW) and age estimations, CFL was converted to straight fork length (SFL; Eqn. 4.1; Parrack & Phares, 1979). Upon landing, dressed weight (DW; body weight minus head, tail, and internal organs) was measured to the nearest kilogram and was converted to total body weight (BW; Eqn. 4.2). When DW was not measured, BW was calculated from SFL based on time of catch according to ICCAT conversion factors (Table. 4.1). All weights and lengths are reported as BW and CFL, respectively, unless otherwise stated. Fish age was estimated from SFL according to Restrepo et al. (2010; Table 4.2).

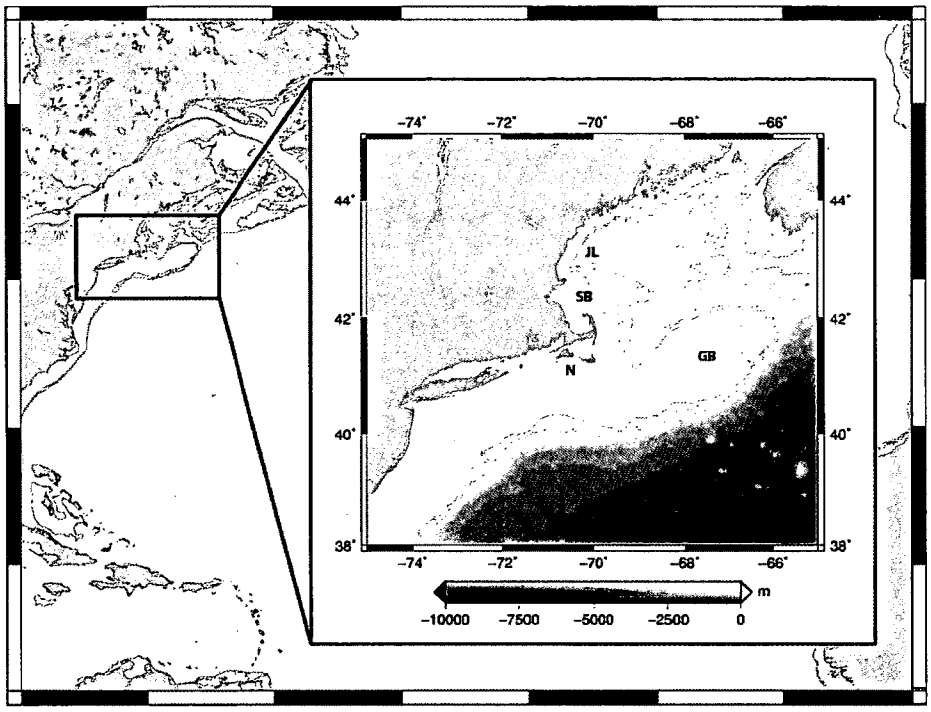

Figure 4-1. Map of the NW Atlantic sampling region. Dotted lines represent the 100m and 200m depth contours, and black and white bands in the border correspond to latitude and longitude. N=Nantucket, GB=Georges Bank, SB=Stellwagen Bank, JL=Jeffrey's Ledge.

$$SFL = CFL \cdot 0.955 \tag{4.1}$$

$$BW = DW \cdot 1.35 \tag{4.2}$$

Histology

Whole gonads and the associated perigonadal fat were dissected from the body cavity immediately upon capture or landing. The perigonadal fat is an adipose fat reserve attached directly to the gonad. This fat reserve is used for general metabolism but is thought to be used primarily for gamete production as fish captured on known spawning grounds with mature gonads have little or no remaining perigonadal fat (Mourente et al., 2002; Corriero et al., 2003; Abascal et al., 2004). The perigonadal fat was removed from the gonad and all tissues (fat and gonad) were weighed separately to the nearest gram. Sex was determined by macroscopic examination of the gonads (Figure 4-2), and the gonadosomatic index (GSI; Eqn 4.3) was calculated using the gonad weight (GW) and BW for each fish. The fat-somatic index (FSI; Eqn 4.4) was also calculated using the weight of the perigonadal fat (FW) and the BW.

$$GSI = 100 \cdot \frac{GW}{BW} \tag{4.3}$$

$$FSI = 100 \cdot \frac{FW}{BW} \tag{4.4}$$

Subsamples (at least 1 cm^3) were excised from the middle of the gonad and fixed in 10% neutral buffered formalin within 24h of collection. Tissue samples were rinsed and stored in 70% ethyl alcohol (EtOH), dehydrated in a series of increasing concentrations of EtOH, and cleared with ClearRite3®. Tissue samples were embedded in paraffin wax, cut to 5 μm sections using a microtome, stained with haematoxylin and eosin, and mounted on glass slides using a high clarity mounting medium. Maturity status for males and females was determined by examining the entire slide using a compound

Month	Body weight conversion
Dec.–March	$BW = 2.861 \cdot 10^{-5} SFL^{2.929}$
April–May	$BW = 6.043 \cdot 10^{-5} SFL^{2.7794}$
June	$BW = 4.404 \cdot 10^{-5} SFL^{2.837}$
July	$BW = 3.733 \cdot 10^{-5} SFL^{2.8683}$
August	$BW = 2.227 \cdot 10^{-5} SFL^{2.9704}$
Sept.	$BW = 1.520 \cdot 10^{-5} SFL^{3.0531}$
Oct.–Nov.	$BW = 0.387 \cdot 10^{-5} SFL^{3.3172}$

Table 4.1. ICCAT conversion equations used to attain body weight (BW) of Atlantic bluefin tuna, *Thunnus thynnus*, when DW was not measured.

Age	SFL	Sdev	Age	SFL	Sdev
0	30.2	1.87	18	257.7	15.90
1	54.5	3.36	19	262.6	16.20
2	76.8	4.74	20	267.1	16.48
3	97.1	5.99	21	271.2	16.73
4	115.7	7.14	22	274.9	16.96
5	132.7	8.19	23	278.3	17.17
6	148.2	9.15	24	281.4	17.37
7	162.4	10.02	25	284.3	17.54
8	175.5	10.83	26	286.9	17.70
9	187.4	11.56	27	289.3	17.85
10	198.2	12.23	28	291.5	17.98
11	208.2	12.85	29	293.5	18.11
12	217.3	13.41	30	295.3	18.22
13	225.6	13.92	31	297.0	18.32
14	233.2	14.39	32	298.5	18.42
15	240.2	14.82	33	299.9	18.50
16	246.6	15.22	34	301.2	18.58
17	252.4	15.57	35	302.4	18.66

Table 4.2. Ages assigned to Atlantic bluefin tuna, *Thunnus thynnus*, based on straight fork length (SFL) according to Restrepo et al. (2010).

Stage	Females	Males
0	Primary oocytes with conspicuous nucleus containing chromatin strands and one large nucleolus	*not defined*
1	Perinucleolar stage oocytes with minor atresia	No evidence of spermatogenesis
2	Vitellogenin independent growth	Spermatocytes
3	Early vitellogenesis	Spermatocytes and spermatids
4	Late vitellogenesis	Mature sperm in small quantities
5	Final oocyte maturation (migratory-nucleus, hydrated, POF)	Fully mature testis (ducts filled with spermatozoa)
6	Degradation and massive atresia	Collapsing ducts and tubules

Table 4.3. Stages assigned to Atlantic bluefin tuna, *Thunnus thynnus*, based on gonad histological characteristics (Heppell & Sullivan, 1999). For both males and females, stages 0–3 are considered immature or non-maturing, stages 4–5 are considered mature-active, and stage 6 is considered mature-inactive.

microscope (40–100x).

Male classification

Histological evidence of recent spawning in male bluefin tuna is only visible for ~12h after spawning (Schaefer, 1998); however spermatozoa are not reabsorbed at the conclusion of the spawning season. If a male does not spawn the milt, it remains in the testis and is visible for months afterwards allowing determination of maturity in individuals sampled far from the spawning grounds. The stages of spermatogenesis differentiated and recorded in maturity determination were, in increasing order of maturity: spermatogonia, spermatocytes, spermatids, and spermatozoa. Male development was classified according to Heppell & Sullivan (1999; Table 4.3) with Santamaria et al. (2003) and Abascal et al. (2004) on hand as references for bluefin tuna specifics. There was no stage 0 for males, and stages 1–3 were considered immature or non-spawning, stages 4–5 were considered mature-active, and stage 6 was considered mature-inactive (Table 4.3).

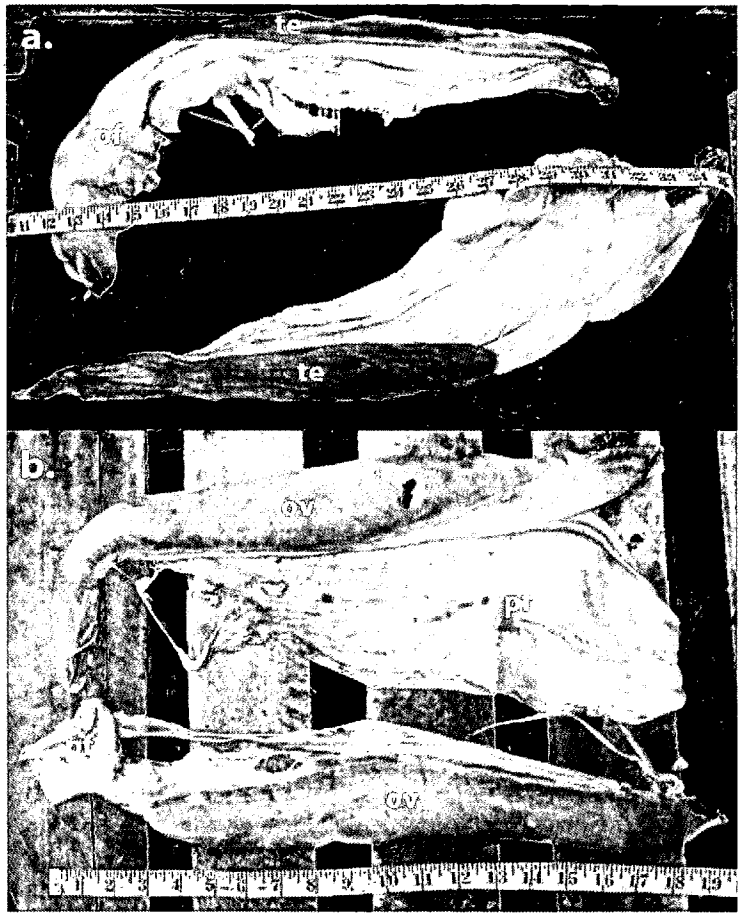

Figure 4-2. Whole gonads from Atlantic bluefin tuna, *Thunnus thynnus*, from the NW Atlantic foraging grounds: (a) male, 380 gm testis and 306 gm perigonadal fat sampled from a 202 cm, 160 kg fish; (b) female, 823 gm ovary and 983 gm perigonadal fat sampled from a 189 cm, 121 kg fish. pf=perigonadal fat, te=testis, ov=ovary.

Female classification

Maturity status for females was assessed by determining the most advanced oocyte stage present in each sample, and stages of development were assigned according to Heppell & Sullivan (1999) to allow comparison with Goldstein et al. (2007; Table 4.3). As with males, stages 0–3 were considered immature or non-maturing, stages 4–5 were considered mature-active, and stage 6 was considered mature-inactive. Tuna specific ovarian characteristics were confirmed according to Schaefer (1998) and Corriero et al. (2003).

When yolked α-atretic follicles are not present, it can be difficult to discern if the fish is immature or mature and in a regressed state after the spawning season (Schaefer, 1998; Corriero et al., 2003). The thickness of the ovarian wall and the presence of copious connective tissue between the lumen has been used as a diagnostic tool for determining the difference between these two reproductive stages in out-of-season winter flounder (*Pseudopleuronectes americanus*; Burton & Idler, 1984) and wahoo (*Acanthocybium solandri*; Maki Jenkins & McBride, 2009). Here, we measured the ovarian wall thickness in at least three locations for each sample, and the average thickness was recorded to determine if this metric can be used as a diagnostic tool for out-of-season bluefin tuna.

Statistics

All statistical analyses were conducted using JMP statistical software (SAS Institute Inc., 1989-2010). Males and females were analyzed separately throughout. For biometric data (CFL, BW, GW, GSI), equal variance was confirmed with the O'Brien test. If variances were equal, Student's t-test was used to compare two means while the Tukey-Kramer HSD test was used for multiple comparisons with Bonferroni corrections for multiple comparisons. If variances were unequal, the Wilcoxon test was

used to compare two groups while the Steel-Dwass test was used for a nonparametric multiple comparisons.

For maturity stage, we used logistic regression to examine the effects of BW, CFL, GW, and month (as an unordered categorical variable; Sokal & Rohlf, 1998; Zar, 1999). We grouped the maturity stages into immature or non-spawning (stages 0–3) and mature (stages 4–6; Goldstein et al., 2007).

Results

From 2004–2011, 981 bluefin tuna were sampled by LPRC scientists from fishing vessels operating in the North Atlantic. Samples were omitted from this study if no gonad was collected, there was no biometric data (length, weight, etc.), or if the date of capture was not recorded. After removing these samples, 510 gonad samples remained (Table 4.4). Our sex ratio was skewed with 60% male fish and 40% female fish.

CFL and age (estimated from length) were 107–292 cm and 3–23 years, respectively (Figures 4-3 and 4-4). Males were significantly older than females (Student's t-test, $p = 0.011$). BW was 21–422 kg (Table 4.5). Since BW was often estimated from SFL, only fish that were weighed were included for BW and length comparisons. Initial statistical analyses revealed significant differences among years for CFL and BW (Steel-Dwass, $\alpha = 0.05$). Beginning in 2008, our sampling from the recreational fishery increased, and therefore, increased the number of smaller fish sampled. When the data were split into two groups (2004–2007 and 2008–2011), the data show no significant difference among years for the 2004–2007 group (Tukey-Kramer HSD, $\alpha = 0.05$). For the 2008–2011 group, there was no significant difference in CFL, but fish sampled in 2008 and 2009 weighed significantly less than those sampled in 2010 and 2011 (Steel-Dwass, $\alpha = 0.05$). This is likely due to an increased number of fish

Size class	CFL range (cm)	Month				
		June	July	Aug.	Sept.	Oct.
School	69 – 119		1	1	1	
Large School	120 – 150	3	5	11	8	
Small Medium	151 – 184	6	5	5	12	
Large Medium	185 – 205	52	49	44	35	7
Giant	> 205	20	67	89	64	25
Total		81	127	150	120	32

Table 4.4. Summary of Atlantic bluefin tuna, *Thunnus thynnus*, gonad samples collected by size class and month in the NW Atlantic foraging grounds. CFL=curved fork length.

sampled from the recreational fishery in 2008 and 2009. For future analyses, the data were split into these two groups, and the years were pooled with the knowledge that 2008 and 2009 contain fish weighing less than those sampled in other years.

For the 2004–2007 group, there was no difference for either male or female CFL or BW among months (Tukey-Kramer HSD or Steel-Dwass, $\alpha = 0.05$). In the 2008–2011 group, females were significantly longer and heavier in October than any other month (Tukey-Kramer HSD or Steel-Dwass, $\alpha = 0.05$). For males in the 2008–2011 group, fish sampled in July and August were significantly longer than those sampled in June or October (Steel-Dwass, $\alpha = 0.05$). Males in this group were significantly heavier in October than in June and September. Males sampled in June were also significantly lighter than those sampled in July and August (Table 4.6; Tukey-Kramer, HSD, $\alpha = 0.05$).

When comparing males to females, there was no significant difference in either CFL or BW for the 2004–2007 group. For the 2008–2011 group, males were significantly heavier in August and were significantly longer in both July and August (Table 4.7; Tukey-Kramer HSD, $\alpha = 0.05$).

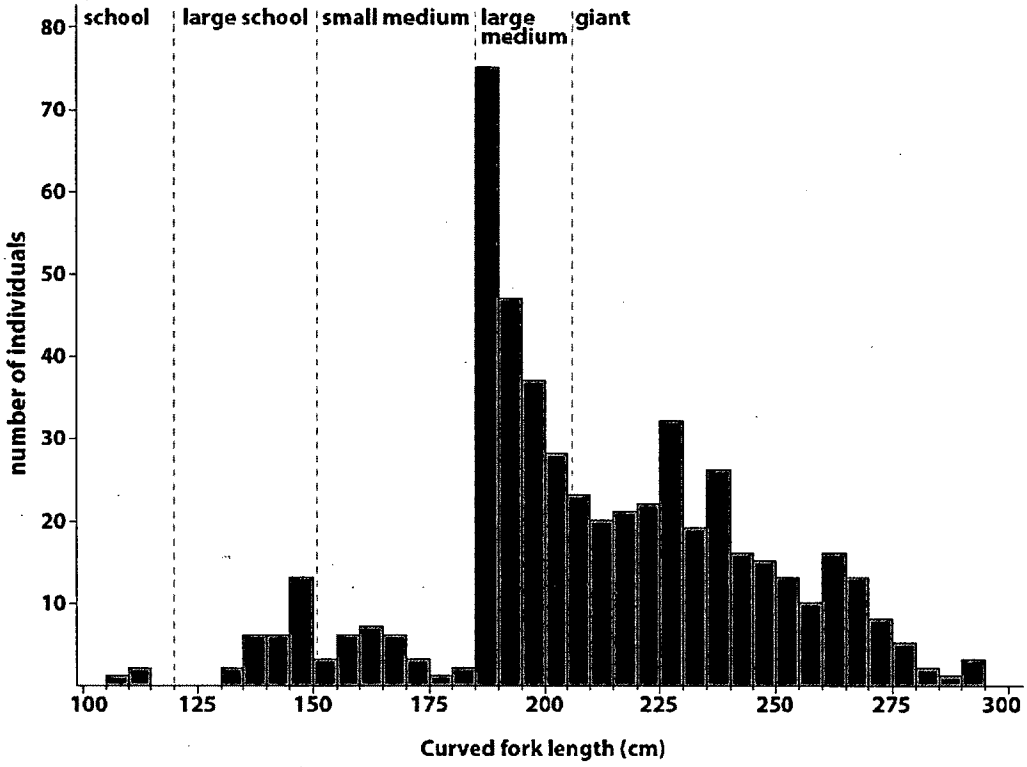

Figure 4-3. Length frequency distribution for Atlantic bluefin tuna, *Thunnus thynnus*, sampled from the NW Atlantic foraging grounds. Dotted lines indicate the beginning of each size class.

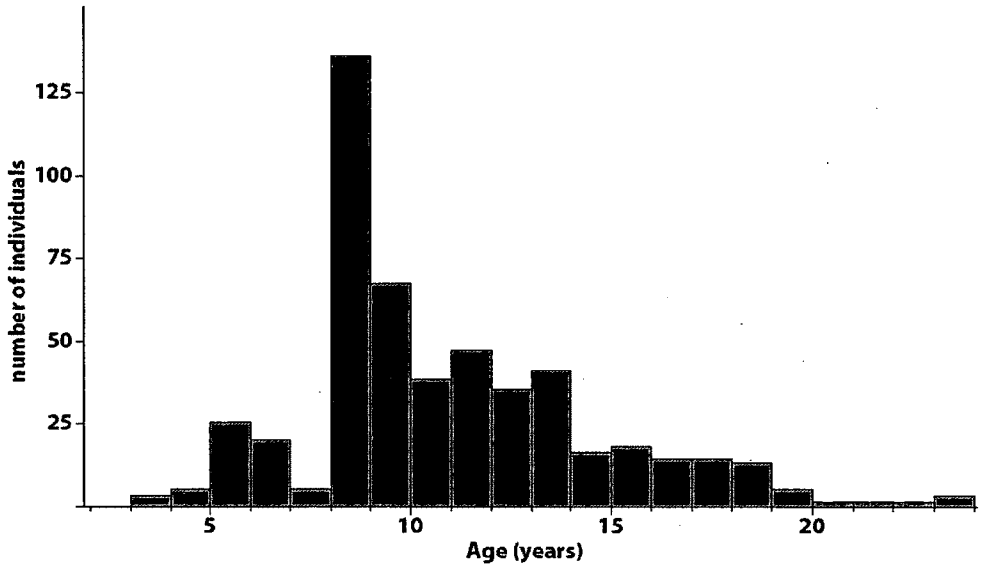

Figure 4-4. Age frequency distribution for Atlantic bluefin tuna, *Thunnus thynnus*, sampled from the NW Atlantic foraging grounds. Ages ranged from 3 to 23 years, with a mean of 10 years and a median of 9 years. Mean age for males was significantly higher (10.7 years) than for females (9.88 years; Student's t-test, $p = 0.011$). All ages reported here were determined based on length according to Restrepo et al. (2010).

Sex	Years		CFL (cm)	BW (kg)	GSI	FSI
Male	2004–2007	$\mu(\pm SD)$	223.3(±24.76)	191.8(±56.95)	0.175(±0.178)	0.514(±0.329)
		Range	150–267	86.58–316.0	0.018–1.068	0.024–1.344
		n	85	87	87	60
	2008–2011	$\mu(\pm SD)$	209.9(±35.07)	163.2(±81.60)	0.173(±0.153)	0.276(±0.237)
		Range	112–292	23.68–401.1	0.016–0.976	0.021–1.160
		n	219	224	224	173
Female	2004–2007	$\mu(\pm SD)$	217.8(±28.16)	182.14(±66.42)	0.430(±0.190)	0.457(±0.306)
		Range	152–282	70.11–342.9	0.065–1.347	0.021–1.015
		n	63	63	63	39
	2008–2011	$\mu(\pm SD)$	199.4(±36.37)	141.3(±76.93)	0.370(±0.223)	0.276(±0.260)
		Range	107–292	21.47–421.8	0.012–1.272	0.005–1.151
		n	143	143	143	99

Table 4.5. Biometric data are summarized for Atlantic bluefin tuna, *Thunnus thynnus*, sampled from the NW Atlantic between 2004 and 2011 for this study. Data are separated into two year groups. CFL=curved fork length; BW=body weight; GSI=gonadosomatic index; FSI=fat-somatic index.

	Month		Score mean diff.	Std-err. diff.	Lower CL	Upper CL	p
BW	7	6	68.174	18.640	16.814	119.534	*0.0030
	7	8	9.035	17.235	-38.453	56.524	0.9848
	7	9	57.532	21.003	-0.338	115.402	0.0522
	8	6	59.139	17.005	12.286	105.992	*0.0056
	8	9	48.496	19.566	-5.414	102.407	0.1000
	9	6	10.642	20.814	-46.707	67.992	0.9862
	10	6	139.039	37.988	34.369	243.710	*0.0030
	10	7	70.865	38.092	-34.091	175.822	0.3426
	10	8	79.901	37.319	-22.926	182.727	0.2074
	10	9	128.397	39.202	20.383	236.411	*0.0109
CFL	7	6	22.154	5.635	4.851	41.237	*0.0008
	8	6	26.945	6.663	7.277	42.450	*0.0005
	8	7	0.263	6.717	-16.980	19.406	1.0000
	9	6	0.400	5.348	-11.345	14.554	1.0000
	9	7	-19.443	5.560	-43.663	-4.851	*0.0043
	9	8	-23.610	6.649	-43.663	-4.851	*0.0035
	10	6	16.856	6.336	-0.554	82.474	0.0600
	10	7	9.147	7.043	-26.759	70.345	0.6921
	10	8	14.179	10.328	-29.108	72.771	0.6451
	10	9	15.476	5.982	-2.426	87.325	0.0727

Table 4.6. Comparison of male curved fork length (CFL) and body weight (BW) among months in the 2008–2011 group. Males weighed significantly less in June than in July, August, and October. Males weighed significantly less in September than in October. Male fish sampled in July and August were significantly longer than those sampled in June and October (Steel-Dwass, $\alpha = 0.05$). $*$ = significance.

	Month	Difference	Std-err. diff.	Lower CL	Upper CL	p
	6	7.187	17.450	-27.675	42.049	0.6818
	7	36.134	22.215	-8.174	80.442	0.1083
BW	8	56.871	20.380	16.437	97.305	*0.0063
	9	17.866	18.039	-18.316	54.048	0.3265
	10	55.931	64.423	-92.627	204.489	0.4106
	6	2.857	5.069	-7.261	12.976	0.5748
	7	16.083	7.455	1.260	30.905	*0.0338
CFL	8	15.820	6.741	2.475	29.165	*0.0206
	9	4.132	7.222	-10.272	18.536	0.5690
	10	11.377	20.817	-35.714	58.467	0.5980

Table 4.7. Comparison of body weight (BW) and curved fork length (CFL) between males and females among months in the 2008–2011 group (Tukey-Kramer HSD, $\alpha = 0.05$). Males were significantly heavier in August and significantly longer in both July and August. $*$ = significance.

Gonad weight (GW) was highly variable, ranging from 8.6 to 2086 gm for the 2004–2007 group and from 3 to 2100 gm for the 2008–2011 group. GSI was 0.018–1.347 for the 2004–2007 group and 0.012–1.272 for the 2008–2011 group (Table 4.5).

For the 2004–2007 group, neither males nor females showed significant differences among months for either GSI or GW (Tukey-Kramer HSD or Steel-Dwass, $\alpha = 0.05$). For the 2008–2011 group, female GSI did not differ among months although GW was significantly higher in October (Steel-Dwass, $\alpha = 0.05$). Male GW was significantly higher in July than in September, and GSI was significantly higher in June than in August and September (Table 4.8; Tukey-Kramer HSD, $\alpha = 0.05$).

When comparing males to females in the 2004–2007 group, females had higher GW in all months except June and July (Tukey-Kramer HSD, $\alpha = 0.05$). Females had significantly higher GSI values than males for every month sampled (Tukey-Kramer HSD, $\alpha = 0.05$). For the 2008–2011 group, females had higher GW and GSI for all months sampled (Tukey-Kramer HSD or Wilcoxon, $\alpha = 0.05$). Overall for both year

groups, females had a higher GW and GSI for all months even though not all months were statistically significant (Figure 4-5).

The perigonadal fat weight (FW) and the fat-somatic index (FSI) were highly variable and ranged from 4–2752 gm and 0.005–1.34, respectively (Table 4.5). For both year groups, FW and FSI increased throughout the sampling period (Figure 4-6).

For the 2004–2007 group, FW was significantly higher in September than in July, but FSI did not differ among months (Steel-Dwass, $\alpha = 0.05$). For males in the 2004–2007 group, FW was significantly lower in June and July than in September and October. FSI was significantly lower in June, July, and August than in both September and October. FSI in August was also significantly higher than in July (Table 4.9; Tukey-Kramer HSD, $\alpha = 0.05$). For the 2008–2011 group, female FW and FSI were significantly higher in September than in June, July, and August (Tukey-Kramer HSD, $\alpha = 0.05$). For males in this group, both FW and FSI were significantly lower in June and July than in August and September (Table 4.10; Steel-Dwass, $\alpha = 0.05$).

When comparing males to females, there were no significant differences in FW or FSI among months in the 2004–2007 group (Tukey-Kramer HSD, $\alpha = 0.05$). For the 2008–2011 group, there were no significant differences in FW or FSI except for in August when males had significantly heavier perigonadal fat than females (Tukey-Kramer HSD or Wilcoxon, $\alpha = 0.05$).

	Month		Difference	Std-err. diff.	Lower CL	Upper CL	p
GW	6	9	138.911	65.163	-40.295	318.117	0.2103
	7	6	52.685	60.829	-114.604	219.974	0.9090
	7	8	44.381	53.571	-102.945	191.707	0.9216
	7	9	191.596	63.164	17.886	365.306	*0.0225
	8	6	8.304	55.913	-145.464	162.072	0.9999
	8	9	147.215	58.445	-13.516	307.945	0.0901
	10	6	175.906	132.194	-187.645	539.458	0.6724
	10	7	123.221	131.221	-237.653	484.095	0.8814
	10	8	167.602	129.015	-187.206	522.411	0.6921
	10	9	314.817	133.285	-51.733	681.367	0.1298
GSI	6	7	0.052	0.030	-0.031	0.134	0.4254
	6	8	0.094	0.028	0.019	0.170	*0.0066
	6	9	0.116	0.032	0.028	0.204	*0.0032
	6	10	0.059	0.065	-0.118	0.237	0.8885
	7	8	0.043	0.027	-0.030	0.116	0.4941
	7	9	0.064	0.031	-0.021	0.150	0.2352
	7	10	0.008	0.064	-0.168	0.184	1.0000
	8	9	0.022	0.029	-0.057	0.100	0.9420
	10	8	0.035	0.063	-0.138	0.208	0.9813
	10	9	0.057	0.065	-0.122	0.235	0.9071

Table 4.8. Comparison of male gonad weight (GW) and gonadosomatic index (GSI) among months in the 2008–2011 group (Tukey-Kramer HSD, $\alpha = 0.05$). GW was significantly higher in July than in September, and GSI was significantly higher in June than in August and September. $*$ = significance.

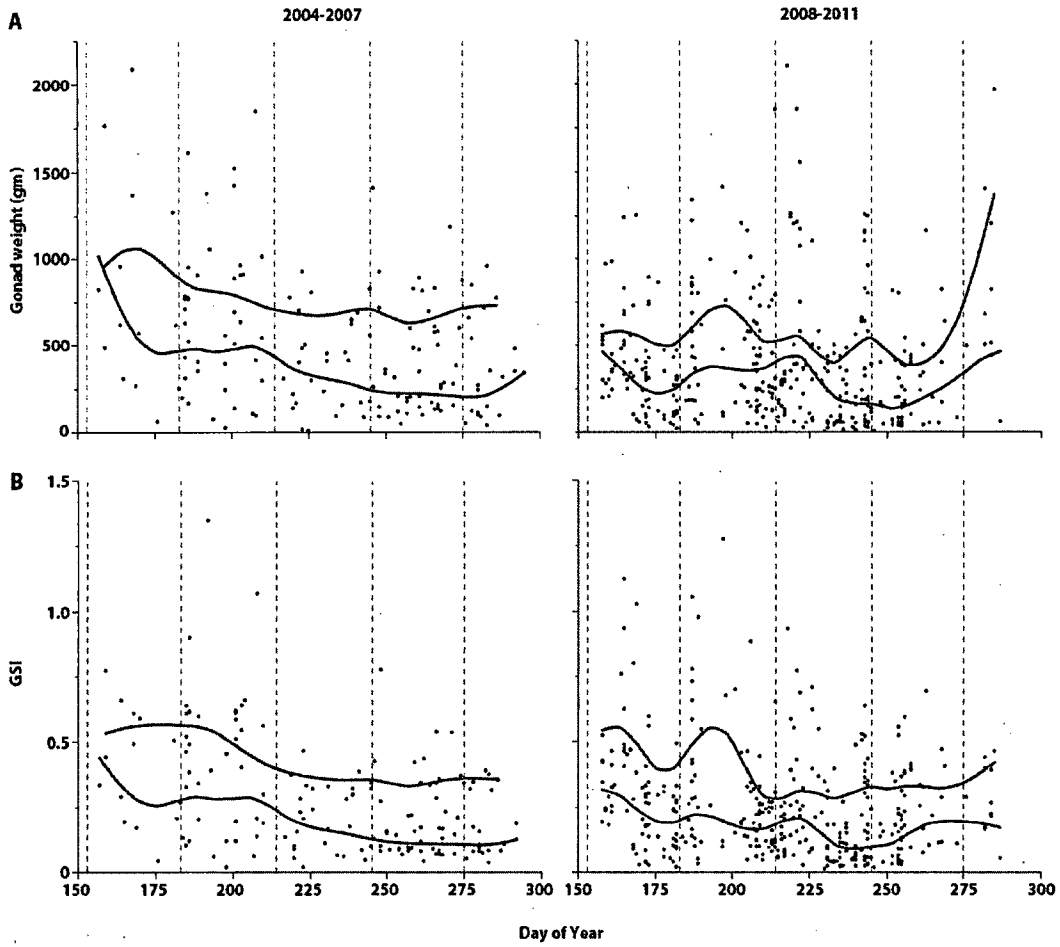

Figure 4-5. Gonad weight (GW; A) and gonadosomatic index (GSI; B) for Atlantic bluefin tuna, *Thunnus thynnus*, over sampling period in the North Atlantic. Dotted lines indicate the beginning of months from June–October. Smooth lines show trends for GW and GSI where blue are males and red are females. Overall, females had higher GW and GSI.

	Month		Difference	Std-err. diff.	Lower CL	Upper CL	p
FW	7	6	163.428	330.395	-766.779	1093.634	0.9876
	8	6	703.944	330.395	-226.263	1634.151	0.2213
	8	7	540.517	227.994	-101.387	1182.421	0.1381
	9	6	1233.571	325.995	315.751	2151.391	*0.0033
	9	7	1070.144	221.570	446.325	1693.962	*<0.0001
	9	8	529.627	221.570	-94.192	1153.445	0.1326
	10	6	1334.429	367.629	299.390	2369.467	*0.0052
	10	7	1171.001	279.234	384.833	1957.170	*0.0009
	10	8	630.484	279.234	-155.684	1416.653	0.1736
	10	9	100.858	274.015	-670.615	872.330	0.9960
FSI	7	6	0.041	0.112	-0.276	0.357	0.9962
	8	6	0.303	0.112	-0.014	0.620	0.0672
	8	7	0.262	0.079	0.038	0.486	*0.0140
	9	6	0.617	0.110	0.306	0.927	*<0.0001
	9	7	0.576	0.076	0.361	0.790	*<0.0001
	9	8	0.314	0.076	0.099	0.528	*0.0012
	9	10	0.021	0.097	-0.253	0.294	0.9995
	10	6	0.596	0.127	0.237	0.955	*0.0002
	10	7	0.555	0.100	0.275	0.836	*<0.0001
	10	8	0.293	0.100	0.012	0.574	*0.0368

Table 4.9. Comparison of male perigonadal fat weight (FW) and fat-somatic index (FSI) among months in the 2004–2007 group (Tukey-Kramer HSD, $\alpha = 0.05$). FW was significantly smaller in June and July than in September and October. FSI was significantly different for a number of monthly comparisons. $*$ = significance.

	Month		Score mean diff.	Std-err. diff.	Lower CL	Upper CL	p
FW	7	6	13.350	5.255	-5.000	187.000	0.0819
	8	6	32.156	6.034	130.000	491.200	*<0.0001
	8	7	21.877	6.166	42.000	375.000	*0.0036
	9	6	21.826	4.761	133.000	540.000	*<0.0001
	9	7	16.471	5.235	33.000	435.000	*0.0143
	9	8	1.010	6.032	-226.000	234.000	0.9998
	10	6	16.985	10.394			0.4754
	10	7	22.989	13.858			0.4597
	10	8	25.898	19.055			0.6539
	10	9	15.455	10.106			0.5433
FSI	7	6	2.943	5.152	-0.046	0.065	0.9792
	8	6	23.564	5.940	0.036	0.246	*0.0007
	8	7	24.693	6.060	0.033	0.220	*0.0004
	9	6	21.333	4.726	0.100	0.399	*<0.0001
	9	7	24.102	5.152	0.103	0.382	*<0.0001
	9	8	10.470	5.940	-0.048	0.260	0.3956
	10	6	16.485	10.108			0.4775
	10	7	21.989	13.282			0.4618
	10	8	30.992	18.478			0.4481
	10	9	16.485	10.108			0.4775

Table 4.10. Comparison of male perigonadal fat weight (FW) and fat-somatic index (FSI) among months in the 2008–2011 group (Steel-Dwass, $\alpha = 0.05$). Both FW and FSI were significantly smaller in June and July than in August and September. $*$ = significance.

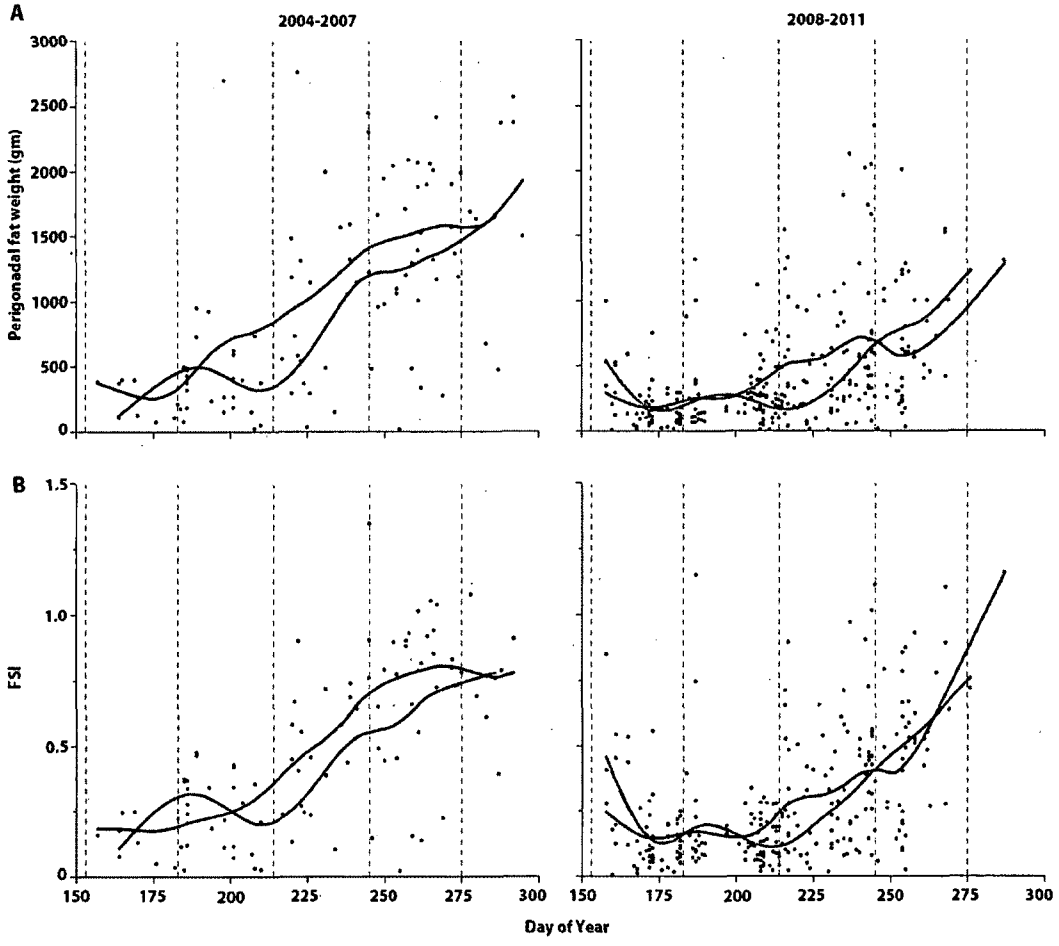

Figure 4-6. Perigonadal fat weight (FW; A) and fat-somatic index (FSI; B) for Atlantic bluefin tuna, *Thunnus thynnus*, over the sampling period in the North Atlantic. Dotted lines indicate the beginning of months from June–October. Smooth lines show trends for FW and FSI where blue are males and red are females. Overall, FW and FSI increased over time.

Histology

For histological results, the data were not separated into year groups as the length and weight of the individuals sampled does not impact the histological descriptions of gonad development. Although gonad weights and GSI values were reported for all samples, histological examination included a total of 323 samples across all years.

Male classification

Males were found in all stages except 0 and 1, and there were no immature or non-spawning males (stages 1–3) sampled in June (Figure 4-7). Many males had ducts with residual spermatozoa or full of spermatozoa indicating maturity (Figure 4-8), and the smallest male to exhibit maturity (stage 4 or higher) was 142 cm with an estimated BW of 48 kg and an estimated age of 5 years (Restrepo et al., 2010). There were seven fish with ages estimated at 5 years that showed mature testes, and only two 5 year old fish that showed immature testes. Despite different ages, these 5 year old fish displayed histological characteristics nearly identical to much older fish whether immature or mature (Figure 4-9). Fish classified as immature (stages 1–3) had estimated ages of 4–21 years, and fish classified as mature (stages 4–6) had estimated ages of 5–19 years (Restrepo et al., 2010).

When considering reproductive stage, both month and GW were significant predictors of reproductive stage ($p < 0.0001$ and $p = 0.0332$, respectively) while SFL and BW were not significant ($p = 0.1578$ and $p = 0.5026$, respectively).

Female classification

Females were observed in all stages except stages 0 and 5 (Figure 4-7). The smallest female to exhibit maturity (stage 4 or higher) was 157 cm with an estimated weight of 66 kg and an estimated age of 6 years (Restrepo et al., 2010). There were nine

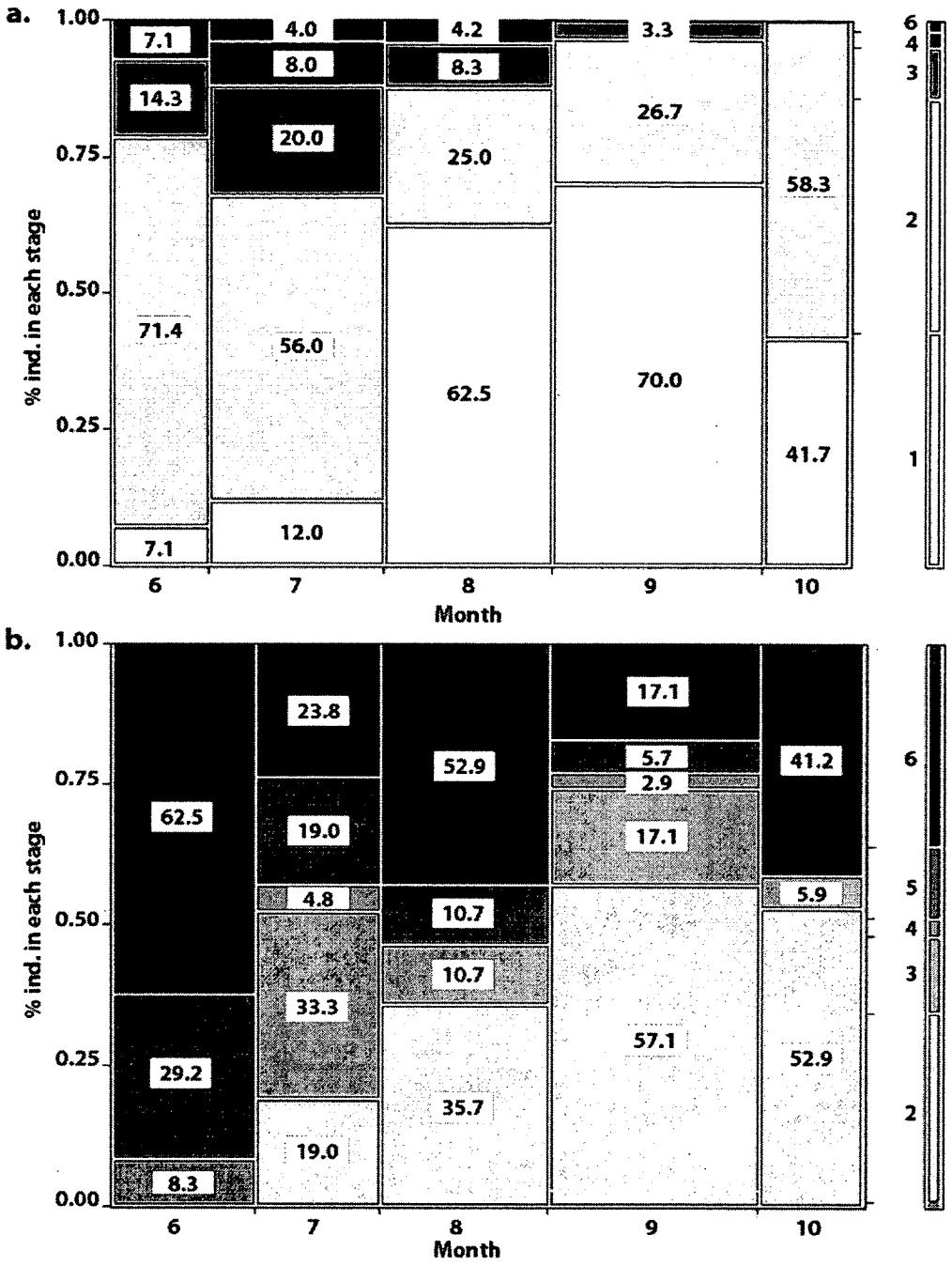

Figure 4-7. Proportion of Atlantic bluefin tuna, *Thunnus thynnus*, classified in each stage of reproductive maturity by sex and month. Different colors correspond to stages of maturity (Table 4.3) as indicated by the bar on the right. (a) females, (b) males; Column widths are representative of sample size (Female: June n=14; July n=25; August n=24; September n=30; October n=12; Male: June n=24; July n=21; August n=28; September n=35; October n=17).

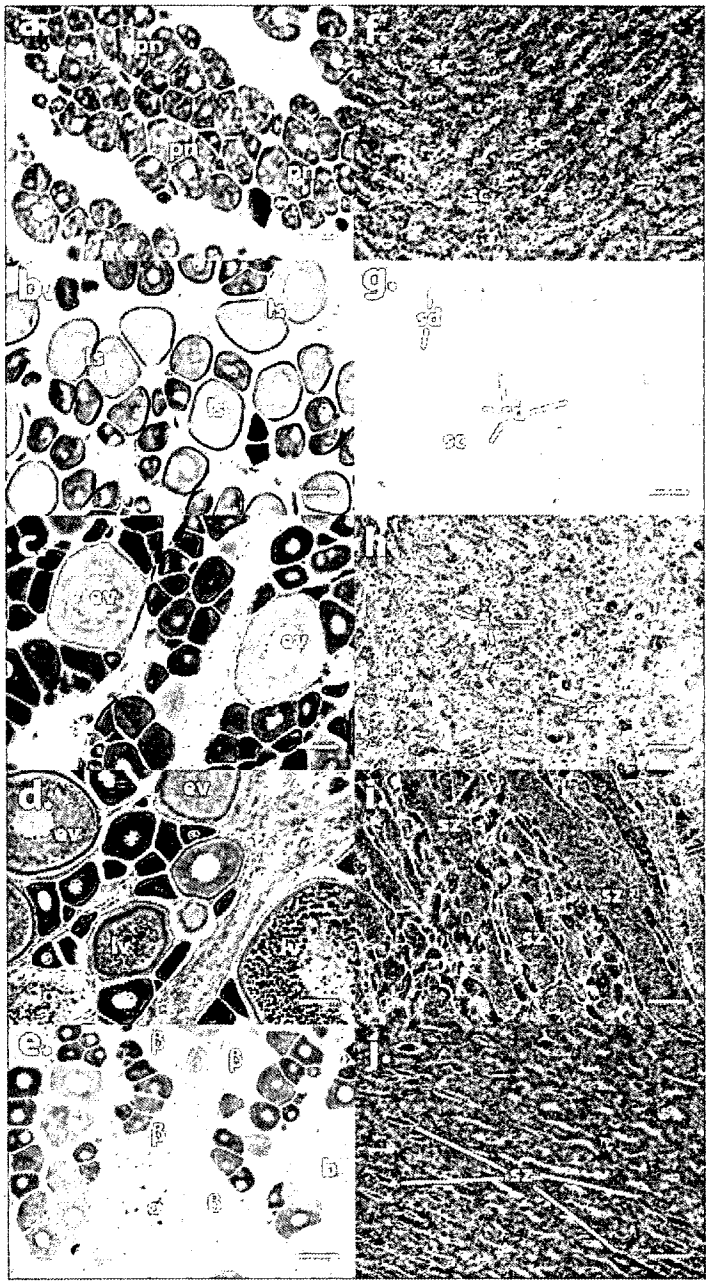

Figure 4-8. Examples of reproductive stages observed in Atlantic bluefin tuna, *Thunnus thynnus*, sampled in the NW Atlantic. (a–e) female fish representing, in order, stages 1–4, and stage 6 (no stage 5 females were observed in this study). (f–j) male fish representing, in order, stages 2–6 (no stage 1 fish were found, and stage 0 was not defined for males). pn=perinucleolar stage, ls=lipid stage (vitellogenin independent growth), ev=early vitellogenic, lv=late vitellogenic, α=α-atresia, β=β-atresia, sc=spermatocytes, sd=spermatids, sz=spermatozoa. Scale bar = 100μm.

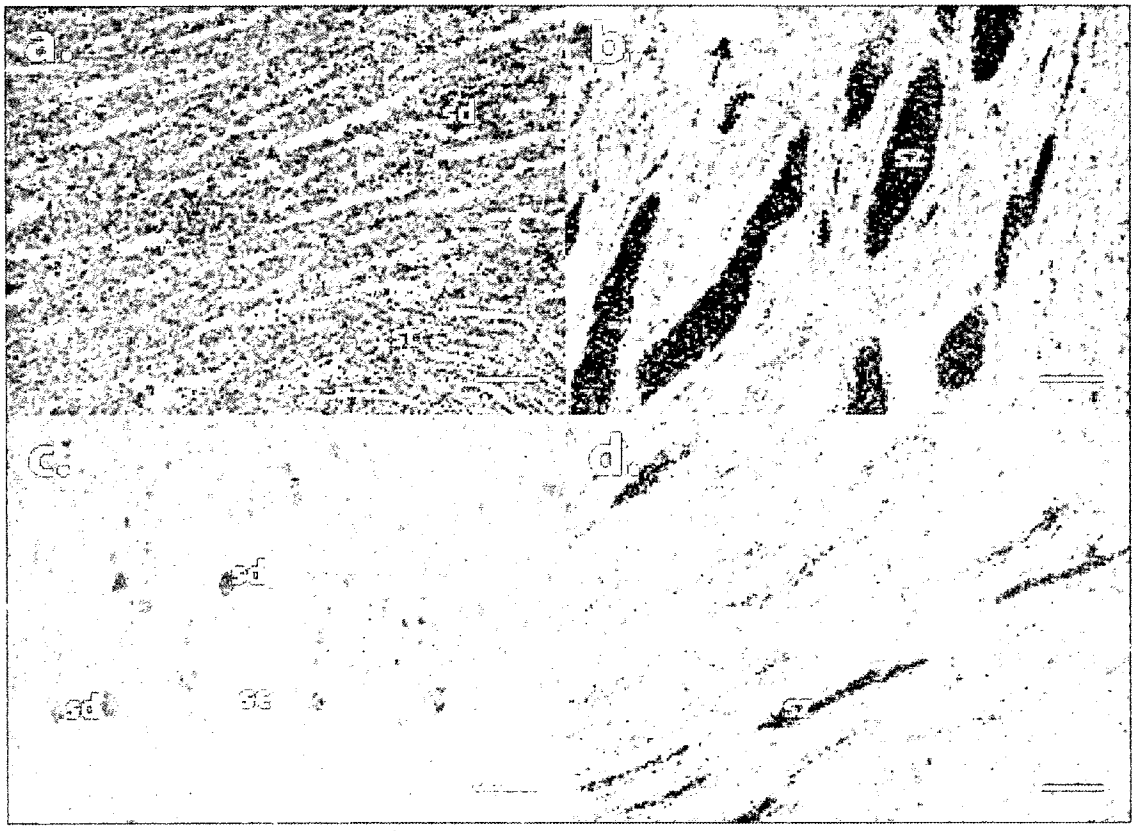

Figure 4-9. Examples of immature and mature male Atlantic bluefin tuna, *Thunnus thynnus*, sampled in the North Atlantic: (a,c) immature fish (stage 3) that are 5 years, 148 cm, 49 kg and 14 years, 249 cm, 269 kg, respectively; (b,d) mature fish (stage 6) that are 5 years, 145 cm, 51 kg and 17 years, 269 cm, 320 kg, respectively. sc=spermatocytes, sd=spermatids, sz=spermatozoa. Scale bar = 100μm.

other 6-year old fish that displayed immature gonad development and only one 6-year old fish with mature gonad development at stage 6 with extensive atresia (Figure 4-10b). Similar to male testis development, ovarian development in young fish often mirrored that of much older, presumed mature fish (Figure 4-10). Females classified as immature (stages 1–3) had estimated ages of 4–23 years, and those classified as mature (stages 4–6) had estimated ages of 8–12 years (Restrepo et al., 2010). Of the 120 females examined for histology, 22.5% (n=27) contained vitellogenic and/or α-atretic oocytes (Figure 4-8c–e), and these fish ranged in estimated age from 6 to 17 years (Restrepo et al., 2010). Because of overall gonad condition, sixteen of these fish were still classified as stages 1 or 2.

The ovarian wall thickness was highly variable but increased as CFL increased. There was a significant difference in the wall thickness between the large school (120–150 cm) and giant (>205 cm) size only (Tukey-Kramer HSD, $p = 0.0015$; Figure 4-11). When considering the amount of connective tissue in the lamellae, the results were highly variable. Both young fish and older fish contained extensive connective tissue, but this was only found in fish classified as stages 1 or 2 (Figure 4-12). Ovarian tissue with extensive connective tissue also contained evidence of maturing oocytes (lipid stage and/or atresia).

When considering reproductive stage, both SFL and GW were significant predictors of reproductive stage ($p < 0.0403$ and $p = 0.0094$, respectively) while month and BW were not significant ($p = 0.5864$ and $p = 0.949$, respectively).

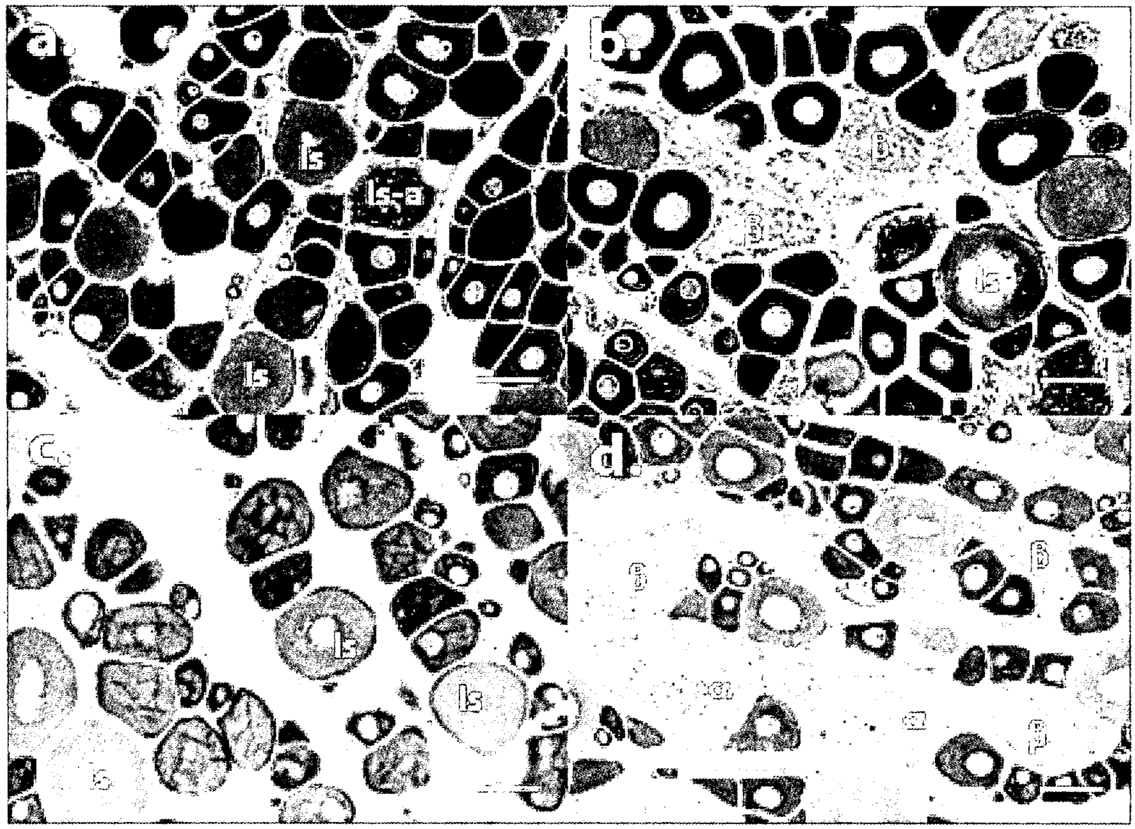

Figure 4-10. Examples of immature and mature female Atlantic bluefin tuna, *Thunnus thynnus*, sampled in the NW Atlantic: (a,c) immature fish (stage 2) that are 4 years, 137 cm, 43 kg and 13 years, 243 cm, 230 kg, respectively; (b,d) mature fish (stage 6) that are 6 years, 157 cm, 66 kg and 12 years, 234 cm, 212 kg, respectively. ls=lipid stage, ls-a=lipid stage atresia, α=α-atresia, β=β-atresia. Scale bar = 100μm.

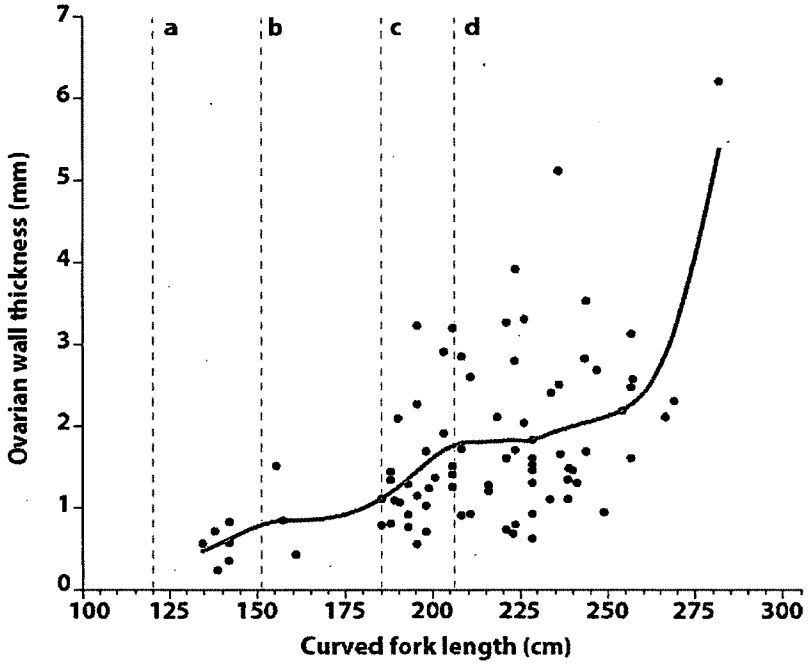

Figure 4-11. Ovarian wall (mm) plotted by curved fork length (CFL; cm). Dotted lines indicate the different size classes: (a) Large School, (b) Small Medium, (c) Large Medium, (d) Giant. Ovarian wall thicknesses of large school and giant fish were significantly different (Tukey-Kramer HSD, $p = 0.0025$).

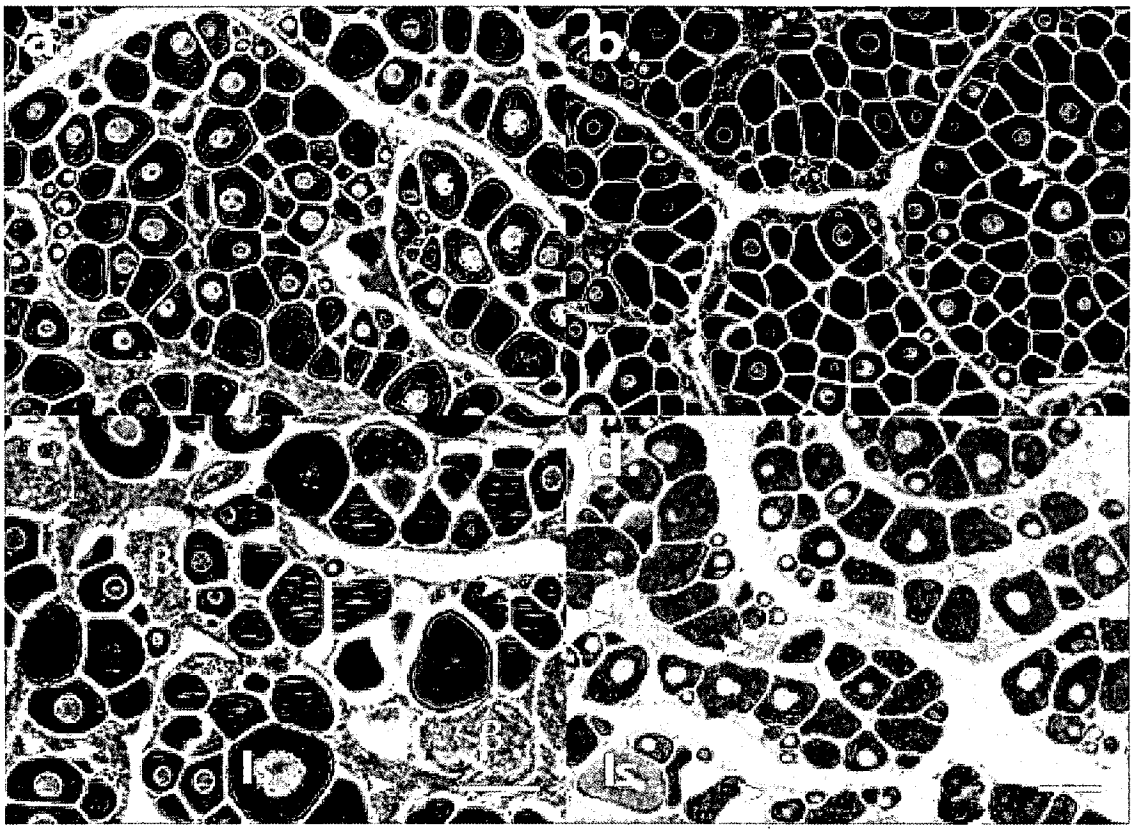

Figure 4-12. Examples of ovarian tissue showing the variability in the amount of connective tissue (marked with arrows). Both old (>9 years) and young (<9 years) fish contained little connective tissue (a,b) and extensive connective tissue (c,d), and fish with extensive connective tissue contained oocytes more mature than perinucleolar stage: (a) 5 years, 139 cm, 46 kg; (b) 18 years, 272 cm, 395 kg; (c) 6 years, 157 cm, 66 kg; (d) 17 years, 267 cm, 313 kg. ls=lipid stage, β=β-atresia. Scale bar = 100 μm.

Discussion

Although our sample lacked fish $\leq$ 107 cm CFL, this is the first study to describe the maturity status of Atlantic bluefin tuna sampled on the northwest Atlantic foraging grounds including fish smaller than the commercially legal size of 185 cm CFL. The sex ratio of female to male fish sampled for this study was 1:1.5, and males in the 2008–2011 group were, on average, larger than females, a result found previously for bluefin tuna (Baglin, 1980, 1982; Goldstein et al., 2007) and yellowfin tuna (Schaefer, 1998). The differences in CFL across months could also be a result of immigration and emigration from the foraging grounds as is found with highly migratory bluefin tuna (Lutcavage et al., 1999; Block et al., 2001; Lutcavage et al., 2001; Stokesbury et al., 2004; Block et al., 2005; Wilson et al., 2005; Rooker et al., 2007; Galuardi et al., 2010). Skewed sex ratios have been previously reported for bluefin tuna with more males present in northern sampling locations (Caddy & Butler, 1976; Goldstein et al., 2007) and more females present in southern sampling locations (Rivas, 1976; Knapp et al., 2012), but this could also reflect a sampling bias associated with relying on fisheries landings.

The trends for gonad weight (GW) and gonadosomatic index (GSI) are consistent with continued oocyte regression and increased body weight throughout the foraging season (Crane, 1936; Dragovich, 1970; Chase, 2002; Estrada et al., 2005; Golet et al., 2007). The significantly higher GW observed in females in October in the 2008–2011 group is likely due to a small sample size for that month (n=5), and all fish sampled in October were 'giants' (>205 cm CFL) while other months had a mix of size classes. Additionally, the trends observed for perigonadal fat weight (FW) and fat-somatic index (FSI) are consistent with increased somatic fat throughout the foraging season (Abascal et al., 2004; Goldstein et al., 2007; Golet, 2010). As bluefin tuna continue foraging on energy rich resources in the NW Atlantic, the somatic fat, perigonadal fat,

and overall condition of the fish increases in preparation for migration and spawning (Crane, 1936; Bigelow & Schroeder, 1953; Dragovich, 1970; Chase, 2002).

Histology

Many bluefin tuna maturity studies focus solely on female fish (Baglin, 1982; Medina et al., 2002; Corriero et al., 2003, 2005; Karakulak et al., 2004b), but because males do not reabsorb unspent milt, they provide invaluable information about the age at maturity especially when combined with information on females (Abascal et al., 2004; Goldstein et al., 2007; Maki Jenkins & McBride, 2009). Despite sampling far from known spawning grounds, many of the males we sampled contained spermatozoa in the testis. The smallest male to show signs of maturity had residual spermatozoa in the ducts, was classified as stage 6, and had an estimated age of 5 years (Restrepo et al., 2010). Of the fourteen male fish examined that were 5–6 years old, only two displayed immature gonads. Of the 120 female fish examined for histology, only six fish were classified as mature (stages 4–6; 5%), but the smallest female to exhibit maturity had extensive β-atresia, was classified as stage 6, and had an estimated age of 6 years (Restrepo et al., 2010).

While only stages 4–6 were considered mature, more than half of all the females sampled contained more than just perinucleolar stage oocytes. The transition from perinucleolar stage to lipid stage follicles (LSF) is the first step in the maturation process, and may be useful in determining maturity in out of season fish. With the exception of three fish, all females sampled in June and July (n=42) contained LSF (93%). However, all individuals lacking LSF had estimated ages over 8 years, and individuals containing LSF had estimated ages from 5–20 years (Restrepo et al., 2010). Additionally, only 15 of the 48 fish sampled in September and October contained LSF (31%), and the individuals containing LSF had estimated ages of 6–18 years

(Restrepo et al., 2010). In Sept. and Oct., both young (<9 years) and old (≥9 years) fish contained perinucleolar stage oocytes as the most advanced stage (Figure 4-13) further confounding the issue of maturity for fish sampled from the foraging grounds. Month appears to be a more accurate predictor of maturity stage than fish size or age for our samples as supported by histology and regression analyses. Corriero et al. (2003) reported 70% of pre-spawning Mediterranean bluefin tuna with lipid stage oocytes as the most advanced stage were of mature size class. The same study showed all fish of immature size sampled between May and September (pre to early post season) with only perinucleolar oocytes as the most advanced stage. All size classes represented in our study contained fish in stage 2 (vitellogenin independent growth/lipid stage follicles) indicating all fish sampled could be mature. As our sample includes 5-year old fish (~132 cm, SFL or ~136 cm, CFL) with evidence of maturity, the lower limit of maturity has not been established for western bluefin tuna. Thus, in order to fully elucidate the maturity ogive for bluefin tuna, research must focus on fish smaller than 130 cm sampled in the western Atlantic.

The use of endocrinological profiles provides an accurate assessment of the reproductive status of fish (Rosenfeld et al., 2012), and for fish sampled far from the spawning grounds, the combination of histology and endocrinology provides the most accurate assessment of their status. Fish sampled from 2008–2011 for histology for this study were also sampled for endocrinology (Heinisch, 2012). Additionally, Heinisch (2012) sampled young of the year (YOY) bluefin tuna for endocrinological analysis of known immature fish. The follicle stimulating hormone (FSH) is the dominant gonadotropin in young fish, and the leutinizing hormone (LH) is dominant for mature fish. When comparing the YOY bluefin tuna to other bluefin tuna sampled, only the YOY fish displayed an FSH/LH ratio consistent with immature fish (≥ 1; Figure 4-14). These findings are consistent with similar analyses of immature and

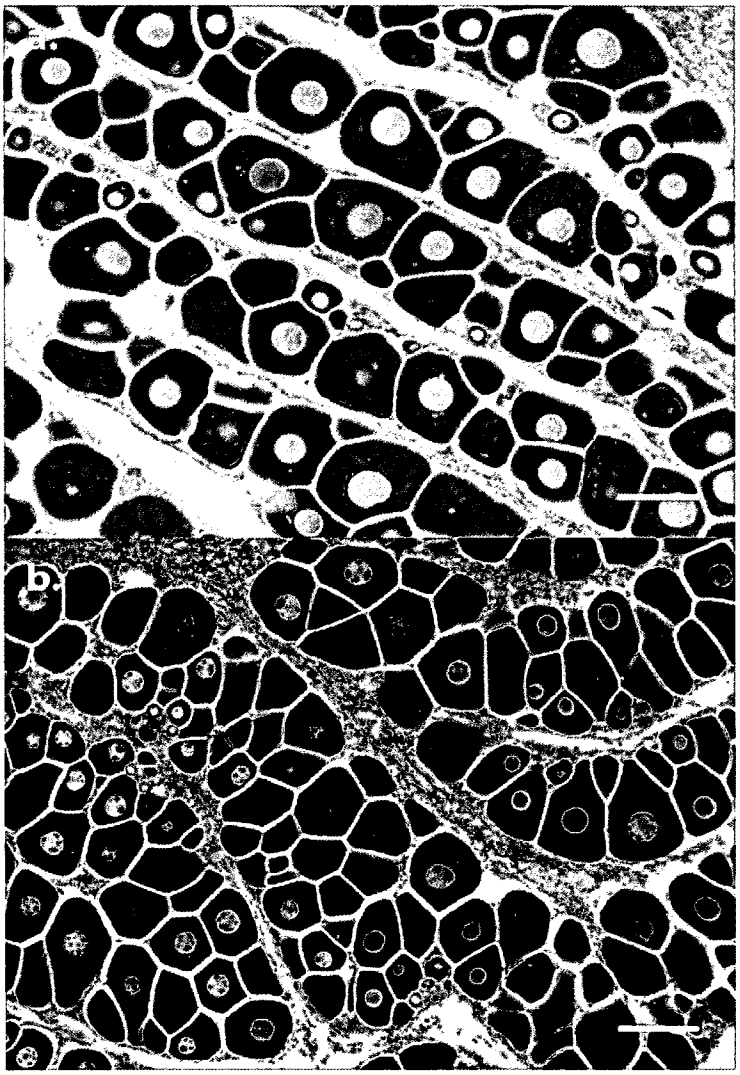

Figure 4-13. Examples of ovarian tissue without lipid stage oocytes from young (a) and old (b) Atlantic bluefin tuna, *Thunnus thynnus* sampled on the NW Atlantic foraging grounds. (a) 138 cm SFL, 45 kg BW, 50 gm GW, estimated 4 years old, sampled on 23 Sept.; (b) 272 cm SFL, 395 kg BW, 1200 gm GW, estimated 18 years old, sampled on 11 Oct. Scale bar = 100 μm.

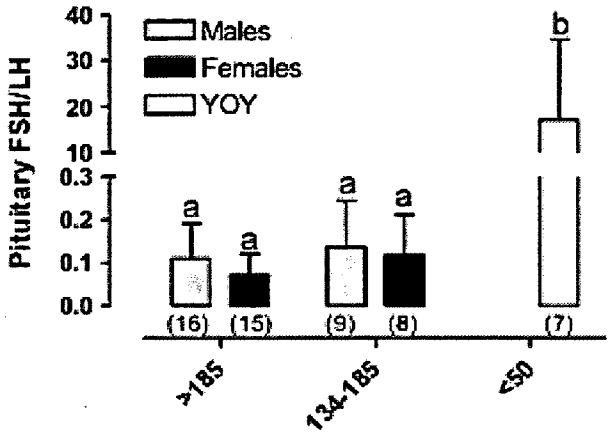

Figure 4-14. Average FSH/LH ratio for Atlantic bluefin tuna, *Thunnus thynnus* sampled in the NW Atlantic and mid-Atlantic Bight (protein levels, μg/pit/BW). The x-axis is straight fork length (cm). Different letters above error bars (std. dev.) indicate significant difference between means (Tukey-Kramer, α=0.05). Sample size is in parentheses. Reproduced with permission from G. Heinisch.

mature bluefin tuna from the Mediterranean (Berkovich et al., *submitted*). For the young fish, we cannot rule out the possibility of a 'dummy-run' as has been observed with young striped bass (*Morone saxatilis*; Holland et al., 2001); however, this behavior only occurs the year prior to actual sexual maturation (Okuzawa, 2002). The histology and endocrinology of the male and female gonads in our sample indicate western bluefin tuna reach maturity between 5–7 years old as suggested by Baglin (1982) and do not support an increase in the age at maturity as recently suggested from length-age estimates (Diaz & Turner, 2007; Anonymous, 2011; Diaz, 2011).

Twenty-three percent of our females sampled had ovaries with α-atretic and vitellogenic stage follicles or early/late vitellogenic oocytes with no atresia (Figure 4-8c–e). Some of these fish, however, were still classified as immature/non-spawning because of the overall ovarian condition. While not included in this study, a re-examination of the samples from Goldstein et al. (2007) revealed 11 out of 79 females (14%; ages

8–12 years) with similar ovarian characteristics (Figure 4-15). Hunter & Macewicz (1985) reported stressed northern anchovy, *Engraulis mordax*, developed atretic follicles within 3 days of being stressed, and all 'healthy' vitellogenic oocytes were atretic within 10 more days. They also found the conversion from α-atretic to β-atretic oocytes lasted 7–8 days for a total of about 21–23 days from no atresia to β-atresia. The rates of oocyte maturation and atresia are temperature dependent and more rapid in warmer conditions (Fitzhugh & Hettler, 1995). Given the high metabolic rate of bluefin tuna (Korsmeyer & Dewar, 2001), it is likely that they reabsorb atretic oocytes much more quickly, and Corriero et al. (2011) found stressed bluefin tuna begin atresia within 24 hours of being stressed. Galuardi et al. (2010) showed a single bluefin tuna migrating from the Gulf of Mexico to the NW Atlantic foraging grounds in about 3 weeks and several making the opposite trip (NW Atlantic to GMX) in no less than 40 days. In a mark-recapture study, a bluefin tuna made a trans-Atlantic migration (7800 km) in about 50 days ($\sim$ 250km/day; Mather et al., 1995). As a conservative estimate, assuming it takes non-stressed bluefin tuna at least 21 days to convert all 'healthy' vitellogenic oocytes into β-atretic oocytes, it is unlikely the fish in our sample containing vitellogenic and/or α-atretic oocytes were spawning in the GMX. These fish were sampled from June to September, and with the known bluefin tuna spawning season in the GMX from April–June (Baglin, 1982), it seems unlikely a fish with vitellogenic oocytes in September had spawned in the GMX in the spring.

These fish could have been spawning outside the GMX along the Gulf Stream edge or in an unknown spawning area (Mather et al., 1995; Lutcavage et al., 1999; Goldstein et al., 2007; Galuardi et al., 2010). Larval surveys have found bluefin tuna larvae outside the GMX (McGowan & Richards, 1989; Muhling et al., 2011a), and ripe or almost ripe fish have been observed outside the GMX during the assumed spawning period (Rivas, 1954; Rathjen, 1961; Wilson & Bartlett, 1967; Mather et al.,

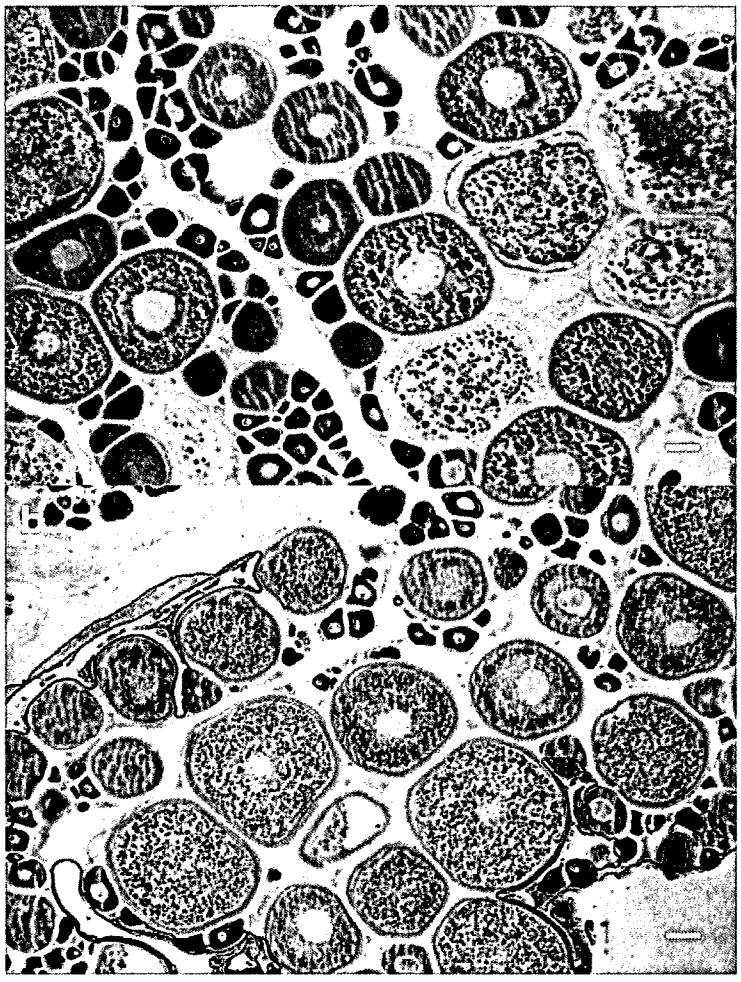

Figure 4-15. Examples of ovarian tissue from samples included in Goldstein et al. (2007) showing both α-atresia (a) and early/late vitellogenesis without any atresia (b). (a) 191 cm, 114.4 kg sampled on 26 June; (b) 179.5 cm, 109.1 kg sampled on 15 July. Both fish have an estimated age of 8 years (Restrepo et al., 2010). Scale bar = 100 μm. Images included with permission of J. Goldstein.

1995). Alternatively, these fish could have skipped spawning (Rideout et al., 2005; Jørgensen et al., 2006; Secor, 2007; Rideout & Tomkiewicz, 2011) though the incidence of skipped spawning in bluefin tuna is unknown. Oocyte maturation represents a significant energetic cost (Kurita, 2003), thus, this scenario is unlikely for large, older fish in a positive energy balance (Chapman et al., 2011). Giant bluefin tuna sampled on the western foraging grounds have extensive perigonadal fat and somatic lipid stores (Goldstein et al., 2007; Golet et al., 2007), and thus seem unlikely candidates for skipped spawning.

An increased ovarian wall thickness has been observed for post-spawning wahoo (*Acanthocybium solandri*; Maki Jenkins & McBride, 2009) and winter flounder (*Pseudopleuronectes americanus*; Burton & Idler, 1987, 1984). Additionally, out of season, mature wahoo were further identified by the presence of copius connective tissue in the lamellae (Maki Jenkins & McBride, 2009). Our sample contained some individuals with increased ovarian wall thickness and connective tissue in the lamellae; however, the lack of trend with fish size, age, or maturity status (Figure 4-12) indicates these characteristics do not appear to be useful identifiers of maturity in Atlantic bluefin tuna. Additionally, the inability to quantify the presence of connective tissue in the lamellae limits its utility as a characteristic of maturity.

The reproductive patterns we observed here for bluefin tuna differ from other tuna species in that other species spawn year-round in the tropical regions of their distributions (e.g., skipjack tuna, *Katsuwonus pelamis*; yellowfin tuna, *T. albacares*; bigeye tuna, *T. obesus*; Matsumoto, 1958; Matsumoto et al., 1984; Nishikawa et al., 1985; Stéquert & Marsac, 1986; Fonteneau & Marcille, 1988; Miyabe, 1994; Stobberup et al., 1998). The migratory tunas (e.g, albacore tuna, *T. alalunga*; southern bluefin tuna, *T. maccoyii*; Pacific bluefin tuna, *T. orientalis*) exhibit reproductive strategies more similar to Atlantic bluefin tuna with migrations to distinct spawning and foraging ar-

eas (Collette & Nauen, 1983; Nishikawa et al., 1985; Stéquert & Marsac, 1986; Caton, 1991; Farley & Davis, 1998; Schaefer, 2001). Additionally, Pacific bluefin tuna have been shown to size segregate on the spawning grounds which could explain the lack of small (< 185 cm, CFL) Atlantic bluefin tuna sampled in the north/central region of the Gulf of Mexico despite our evidence for a younger age at maturity. Southern bluefin tuna are found on their spawning grounds throughout the year with peaks in abundance during the known spawning season (Farley & Davis, 1998), and recent electronic tagging research shows Atlantic bluefin tuna entering the Gulf of Mexico as early as November (Galuardi et al., 2010). While some speculate that the warm temperatures associated with spawning areas are thermally stressful for endothermic Atlantic and Pacific bluefin tuna (NRC, 1994; Blank et al., 2004; Landeira-Fernandez et al., 2004), an early entrance to the Gulf of Mexico may be indicative of continued foraging prior to spawning. Recent electronic tagging research questions the assumptions of annual spawning and spawning periodicity in southern bluefin tuna (Evans et al., 2012), and without further sampling and electronic tagging, these questions also remain unresolved for Atlantic bluefin tuna.

Our results suggest that further sampling across the entire migration range, including the foraging grounds and potential alternative western Atlantic spawning grounds (Lutcavage et al., 1999; Galuardi et al., 2010), is necessary to fully investigate the reproductive potential of western Atlantic bluefin tuna. Although we can predict potential spawning areas based on spatially and temporally explicit results from electronic tagging (Lutcavage et al., 2012) and oceanographic profiling (Teo et al., 2007a; Galuardi et al., 2010), this has proven difficult (Lutcavage & Luckhurst, 2001) as logistics and US regulations on bluefin tuna retention make offshore spawning areas hard to sample. Since we suspect offshore longline fisheries only sporadically encounter spawning bluefin tuna, and tagging results (Galuardi & Lutcavage, 2012;

Lutcavage et al., 2012) and larval habitat modeling (Lehodey et al., 2006; Muhling et al., 2011b) suggest potential Atlantic spawning areas are likely to change, the evaluation of western spawning and larval development areas will require far more extensive sampling.

Finally, continued sampling on the western Atlantic foraging grounds is necessary to fully describe the western bluefin tuna maturity ogive. Special emphasis should be placed on acquiring samples from fish aged 3–5 years as there is a dearth of information about western bluefin tuna of this size/age. Additionally, 44–60% of fish in this size range (102–138 cm, CFL) sampled in the western Atlantic are of Mediterranean origin (Rooker et al., 2008a,b), and thus, comprehensive biological sampling (gonads, otoliths, pituitaries, etc.) is necessary to determine natal origin and an accurate maturity schedule for western Atlantic bluefin tuna.

CHAPTER 5

CONCLUSIONS

This dissertation research represents the first attempt to quantitatively portray the maturity schedule of western Atlantic bluefin tuna, *Thunnus thynnus*, using direct histological examination of gonad tissue collected both on and off the known spawning grounds. Bluefin tuna are highly migratory and have high thermal tolerances (Carey & Teal, 1969; Carey & Lawson, 1973) allowing them to forage throughout the North Atlantic Ocean (Lutcavage et al., 1999; Block et al., 2001; Lutcavage et al., 2001; De Metrio et al., 2005; Wilson et al., 2005; Galuardi et al., 2010; Galuardi & Lutcavage, 2012); however, spawning is believed to occur in only in the Mediterranean Sea, the Gulf of Mexico, and the Straits of Florida (Rivas, 1954; Tiews, 1963; Rodríguez-Roda, 1964; Mather et al., 1995; Nemerson et al., 2000; Susca et al., 2001b,a; Medina et al., 2002; Block et al., 2005; Corriero et al., 2005).

To understand the reproductive dynamics of the western spawning stock, comprehensive size sampling must occur both on and off the spawning grounds. Sampling fish on the spawning grounds provides information about reproductive traits that are only detectable from actively spawning fish, such as fecundity, spawning periodicity, and spawning frequency (Medina et al., 2002; Corriero et al., 2005; Medina et al., 2007; Aranda et al., 2012). Because the spawning locations of western bluefin tuna are still unresolved (Lutcavage et al., 1999; Goldstein et al., 2007; Teo et al., 2007b; Boustany et al., 2008; Galuardi & Lutcavage, 2012; Lutcavage et al., 2012), sampling from the foraging grounds provides a more comprehensive size sample that better represents the entire stock. Previous studies examining bluefin tuna reproduction

focused on female fish (Baglin, 1982; Medina et al., 2002; Corriero et al., 2003, 2005; Karakulak et al., 2004b); however, male fish provide information about maturity long after the spawning season as milt is not reabsorbed (Abascal et al., 2004; Goldstein et al., 2007; Maki Jenkins & McBride, 2009). We showed that males and females exhibit fully mature gonads on the spawning grounds, but females provided more information about spawning periodicity. Alternatively, when sampling off the spawning grounds, male fish provided more information about maturation than females.

The migration patterns and extensive mixing of the two stocks have been documented through numerous electronic tagging and genetic studies (Lutcavage et al., 1999, 2001; Block et al., 2001, 2005; De Metrio et al., 2002; Pujolar et al., 2003; De Metrio et al., 2005; Wilson et al., 2005; Teo et al., 2007a; Rooker et al., 2008a; Dickhut et al., 2009; Galuardi et al., 2010; Medina et al., 2011; Galuardi & Lutcavage, 2012). As the eastern stock is larger than the western stock, the resulting mixing rates are unbalanced with the eastern stock having greater influence on the western population; consequently, any management action aimed at the eastern stock may indirectly affect the western stock (Rooker et al., 2008a). Therefore, understanding the similarities and differences in the reproductive potential of both stocks is necessary as this influences recruitment and the sustainability of the stocks and their capacity for supporting commercial fisheries (Baglin, 1982; Mather et al., 1995). This study documented that the fecundity of the western spawning fish is lower than that of the eastern spawning stock despite the significantly larger size of the western spawning fish sampled in the Gulf of Mexico. We also showed that eastern and western bluefin tuna exhibit the same spawning periodicity (three months), but spawning in the northern Gulf of Mexico occurs one month earlier than in the western Mediterranean spawning ground. The spawning condition of Mediterranean spawners from mid-June to mid-July is comparable with the reproductive peak observed in the Gulf

of Mexico in May. The assumed younger maturation age observed in the eastern population (Rodríguez-Roda, 1967; Baglin, 1982; Medina et al., 2002; Corriero et al., 2005) leads to higher spawning rates and larger productivity in the eastern stock than in the western stock. However, comprehensive spatial and temporal sampling of the western spawning stock has not been conducted and, consequently, the extent of the spatial distribution of this spawning stock remains uncertain.

Our results support historical findings of an age at maturity for western bluefin tuna of 4–6 years (Westman & Neville, 1942; Baglin, 1982) and do not support recent proposed increases in the age at maturity based on landings and electronic tagging data (Diaz & Turner, 2007; Teo et al., 2007a; Diaz, 2011). In our sample, 5-year old male gonad samples collected from the foraging grounds had spermatozoa in the collecting ducts indicating maturity and potentially, previous spawning. Additionally, 6-year old female gonad samples collected from the foraging grounds had vitellogenic and/or α-atretic oocytes, indicating maturity. Given the high metabolic rate of bluefin tuna (Korsmeyer & Dewar, 2001) and known migration speeds of 4–8 kts (Lutcavage et al., 2000), it is unlikely that these females, sampled in September, were spawning in the north/central region of the Gulf of Mexico in the spring. These histological results are supported by endocrinological profiles showing similar levels of the follicle stimulating and leutinizing hormone levels in medium and large bluefin tuna (141–194 cm and >194 cm, CFL, respectively; Heinisch, 2012).

My overall dissertation objective was to revise the knowledge of the reproductive biology of western Atlantic bluefin tuna. Here, we provided evidence for sexual maturation in western bluefin tuna with estimated ages of 5–35+ years (Restrepo et al., 2010). While not spatially or temporally comprehensive, these results call for a revision of the western bluefin tuna maturity schedule. Additionally, continued research on the reproductive biology of the western Atlantic bluefin tuna is warranted

with special attention given to fish approaching maturity with estimated ages of 3–5 years. Until the temporal and spatial variability of spawning for western bluefin tuna is resolved, more sampling off the known spawning grounds is crucial.

Mather et al. (1995) said, "Considering the amount of research which has been devoted to the Atlantic bluefin tuna, positive information on its spawning habits is surprising incomplete." The results of this study have greatly increased the knowledge of the reproductive biology of western Atlantic bluefin tuna and have identified specific priority research areas. With increased spatially and temporally comprehensive biological sampling and with advancing electronic tagging technology, a more comprehensive understanding of reproduction in western Atlantic bluefin tuna is now within reach and, so far, confirms historical findings on maturity status.

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