NEW RESULTS ON MATURITY STATUS OF WESTERN ATLANTIC BLUEFIN TUNA, *THUNNUS THYNNUS*

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SUMMARY

We analyzed the reproductive and sexual maturity of 529 Atlantic bluefin tuna (ABFT, Thunnus thynnus*) sampled from 2004-2010 on NW Atlantic foraging grounds off New England, Canada, and (young of the year, YOY) Virginia. Fish size was 107-292 cm CFL (excluding YOY), and gonadosomatic index (GSI) was 0.012-1.347. Although nearly all gonads sampled from fish >134 cm were regressed, sexual maturity evidence was detected via histology. Partially spent testes were present in males >145 cm and lipid stage oocytes were present in most females sampled in the Gulf of Maine during June-July. We obtained endocrine hormone profiles and compared pituitary gonadotropins (GtHs) across size classes, including YOY, presumably immature, and mature individuals. FSH/LH ratio was >2 among YOY (characteristic of immature fish) while FSH/LH ratio was <1 in ABFT >134 cm (characteristic of mature fish). Although some size gaps remain in our sampling (e.g., between YOY and 107 cm), our results are consistent with histological and endocrine analyses of maturity patterns in eastern ABFT and suggest a revision of the western ABFT maturity schedule is warranted.*

RÉSUMÉ

Nous avons analysé la maturité sexuelle et reproductive de 529 spécimens de thon rouge de l'Atlantique (ABFT, Thunnus thynnus*) échantillonnés entre 2004 et 2010 dans des zones d'alimentation de l'Atlantique Nord-Ouest au large de la Nouvelle-Angleterre et du Canada ainsi que de juvéniles de l'année (YOY) de Virginie. La taille des poissons oscillaient entre 107 et 292 cm CFL (sauf les YOY) et l'indice gonadosomatique (GSI) s'élevait à 0,012-1,347. Même si presque toutes les gonades prélevés de poissons > 134 cm étaient en étape de régression, preuve de la maturité sexuelle détectée par le biais de l'histologie. Des testicules partiellement vides étaient présents chez les mâles >145 cm et des ovocytes au stade lipide étaient présents dans la plupart des femelles échantillonnées dans le golfe du Maine entre juin et juillet. Nous avons obtenu des profils hormonaux endocriniens et avons comparé les gonadotrophines pituitaires (GtHs) entre les classes de taille, y compris les YOY, vraisemblablement immatures, et les spécimens matures. Le ratio FSH/LH était >2 entre les YOY (caractéristique des poissons immatures) tandis que le ratio FSH/LH était <1 chez les ABFT >134 cm (caractéristique des poissons matures). Même si quelques lacunes demeurent au niveau des tailles dans notre échantillonnage (p.ex. entre les YOY et 107 cm), nos résultats concordent avec les analyses histologiques et endocriniennes des schémas de maturité chez les ABFT de l'Est et suggèrent qu'il est justifié de réviser le schéma de maturité des ABFT de l'Ouest.*

RESUMEN

Se analiza nueva información sobre madurez sexual y reproductiva de 529 atunes rojos (ABFT, Thunnus thynnus*) muestreados desde 2004 hasta 2010 en las zonas de alimentación del Atlántico noroccidental en aguas de Nueva Inglaterra y Canadá y juveniles del año (YOY) de Virginia. La talla de los peces era de 107-292 cm CFL (excluyendo a los YOY) y el índice gonadosomático (GSI) era de 0,012-1,347. Aunque casi todas las gónadas muestreadas de peces >134 cm estaban en etapa de regresión la prueba de madurez sexual se detectó mediante histología. Machos > 145 cm presentaban testículos parcialmente vacíos, y la mayoría de las hembras muestreadas en el golfo de Maine durante junio-julio presentaban ovocitos en etapa lipídica. Se obtuvieron perfiles endocrinos de hormonas y se compararon las gonadotropinas de la pituitaria (GtHs) entre las clases de talla, incluido entre los YOY, presumiblemente inmaduros, y ejemplares maduros. La ratio FSH/LH era >2 entre los YOY (característico de*

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peces inmaduros) mientras que la ratio FSH/LH era <1 en atunes rojos del Atlántico >134 cm (característico de peces maduros). Aunque sigue habiendo algunas lagunas de talla en nuestro muestreo (por ejemplo entre YOY y 107 cm), nuestros resultados son coherentes con análisis histológicos y endocrinológicos de patrones de madurez del atún rojo del Atlántico oriental y sugieren que merece la pena hacer una revisión del calendario de madurez del atún rojo del Atlántico occidental.

KEYWORDS

Atlantic bluefin tuna, sexual maturity, gondotropins, histology, NW Atlantic

1. Introduction.

The age of maturity for Western Atlantic bluefin tuna (ABFT) has remained unresolved for decades (Mather *et al*. 1995, Goldstein *et al*. 2007, Diaz 2011). Historic studies determined the age of maturity was 5–6 y based on macroscopic examination of gonads obtained from fish sampled via long line fishing off New England and the mid–Atlantic shelf (Wilson 1965, Baglin 1982). In contrast, individuals sampled via long line fishing in the Gulf of Mexico, a known spawning ground, were considerably larger/older (8–12 y; Richards 1976, Baglin 1982, Diaz 2011). The ICCAT Bluefin Program has repeatedly called for additional research on reproductive biology, and in 1999, recommended maintaining a sample archive. The age and size at maturity of western ABFT have significant bearing on stock assessment, yet the western ABFT maturity ogive remains controversial. A number of historic studies suggested western ABFT mature between the ages of 5–7 y (Westman and Neville 1942, Wilson 1965, Baglin 1982, Locke 1995, Mather *et al*. 1995), with some fish maturing at age 4 (Westman and Neville 1942, Mather *et al*. 1995). However, 9 y is the age currently assumed for western ABFT sexual maturation (ICCAT 2010;), and ages at maturity of 12–16 y have been proposed (Diaz and Turner 2007, Diaz 2011). These maturity schedules are strikingly different from eastern ABFT (Tiews 1963, Rodriguez-Roda 1967, Susca *et al*. 2000, Karakulak *et al*. 2004, Corriero *et al*. 2005) despite recent determination of similar growth curves (Restrepo *et al*. 2010). Since reproduction is a major inhibitor of growth in teleosts, western ABFT should exhibit faster growth rates than the presumed earlier maturing eastern ABFT. 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*. 2008, Dickhut *et al* 2009).

Since 2000, the Large Pelagics Research Center (LPRC) has conducted biological sampling of fish landed by the US and Canadian commercial fisheries in the Gulf of Maine and adjacent SW Nova Scotia shelf, respectively, during summer and fall, and previously reported on the maturity status of individuals landed (≥185 cm CFL) via the U.S. commercial fisheries (Goldstein *et al*. 2007). In 2008, we began more extensive sampling that included smaller fish targeted by the U.S. recreational fishery.

While sampling tunas on the spawning grounds provides valuable information about the spawning stock (Schaefer, 1998), sampling on the foraging grounds provides a more comprehensive size sample more representative of the entire stock (Fromentin and Powers 2005). However, as ABFT have a high metabolic rate and reabsorb signs of maturity (post-ovulatory follicles, vitellogenic oocytes, etc.) quickly, utilizing foraging fish for reproductive studies requires the use of indirect methods for determining reproductive status. 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). The use of endocrinological profiles provides an accurate assessment of the reproductive status of fish (Rosenfeld *et al*. 2012; Berkovich *et al*. submitted), and for fish sampled far from the spawning grounds, the combination of histology and endocrinology provides the most accurate assessment of their status.

To better understand ABFT sexual maturity schedules and to investigate the discrepancy between the ages at maturity of the two spawning stocks, we sampled ABFT over a range of sizes in the NW Atlantic, presumably representing immature and mature fish. We utilized histology and endocrinology and compared results with studies applying similar techniques for Mediterranean ABFT to assess the maturity status of western ABFT.

2. Materials and Methods

Samples of male and female ABFT gonads were collected between 2004 and 2011 from the Gulf of Maine and SW Nova Scotia (NW Atlantic) foraging grounds. Young of the year (YOY) tuna were also caught and retained under an Exempted Fishing Permit (NMFS_TUNA-EFP-08-03). For endocrine analyses, pituitary glands were immediately dissected from the head and placed in liquid nitrogen or in dry ice on board. Endocrine samples were stored in liquid nitrogen until processing. Curved fork length (CFL) was measured to the nearest centimeter and upon landing, dressed weight (DW) was measured to the nearest kilogram and was converted to total body weight (BW),

$$BW=DW \cdot 1.35$$

When DW was not measured, BW was calculated from CFL based on time of catch according to ICCAT conversion factors. All weights and lengths are reported as BW and CFL, respectively, unless otherwise stated. Fish age was estimated by based on fish length (converted to SFL) according to Restrepo *et al*. (2010).

Whole gonads and the associated perigonadal fat were dissected from the body cavity immediately upon capture or landing. The perigonadal fat was removed from the gonad and the gonad was weighed to the nearest gram. The Gonadosomatic Index (GSI) was calculated using the gonad weight (GW) and BW for each fish,

$$GSI = (GW/BW) \cdot 100$$

Subsamples were excised from the middle of the gonad and fixed in 10% neutral buffered formalin within 24 h 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 both males and females was determined by examining the entire slide using a compound microscope (40–100x).

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 and Sullivan (1999) to allow comparison with Goldstein *et al*. (2007). 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).

Histological evidence of recent spawning in males is only visible for about 12 h after the spawning event (Schaefer 1998); however spermatozoa are not reabsorbed after the spawning season. If a male does not spawn the milt, it remains in the testis and is present for months afterwards, allowing determination of maturity far from the spawning grounds. Male development was classified according to Heppell and Sullivan (1999) with Santamaria *et al*. (2003) and Abascal *et al*. (2004).

Pituitary leutinizing hormone (LH) levels were measured using an ELISA developed for striped bass (Mananos *et al*. 2002) and modified for ABFT (Rosenfeld *et al*. 2003, Rosenfeld *et al*. 2012). Pituitary follicle stimulating hormone (FSH) was measured using a quantitative dot blot as follows: 20 µl of each sample (diluted 1:2 to 1:10) and 1:2 standard curve dilution (2–0.0625 µg/ml) were loaded on a nitrocellulose membrane (Whatman, Maidstone, UK). The membrane was blocked in 10% skim milk then incubated at room temperature for one hour with primary antibody (1:10,000 anti ABFT FSH). Following five washes in assay buffer (PBS-T), the membrane was incubated (room temperature, in the dark) for another hour with a secondary antibody (1:5,000 GAR-HRP, Bio-Rad) followed by a second wash cycle. The membrane was then incubated for 5 min at room temperature in an ECL solution (SuperSignal, Thermo Scientific, Waltham, MA). Results were obtained and analyzed using G-Box and Gene Tools (Syngene, Cambridge, UK).

Data were analyzed using JMP 9 statistical software (SAS Institute Inc., Cary, NC). We used ArcSine transformation to normalize the GSI distribution. For all parameters normal distributions were confirmed using Shapiro-Wilk *W* test for Goodness of fit. One-Way ANOVA followed by *Tukey-Kramer* HSD ($\alpha = 0.05$) was used to determine significant differences between means (Sokal and Rohlf 1995). Parameters in figures and text are presented as means ± SD.

3. Results and Discussion

From 2004–2011, LPRC scientists sampled a total of 981 bluefin tuna from fishing vessels operating in the NW 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, 529 gonad samples remained. Our sex ratio was skewed with 60% male fish and 40% female fish. CFL for YOY fish ranged from 12–37 cm. CFL for all others ranged from 107–292 cm, and GSI ranged from 0.012–1.4 (**Table 1**). Many males had ducts containing spermatozoa (**Figure 1**). The smallest male to exhibit maturity (stage 4 or higher) was 142 cm with an estimated BW of 48 kg and contained residual spermatozoa in the testes. According to Restrepo *et al*. (2010), this fish was 5 y old. Females were observed in all but stages 0 and 5 (**Figure 2**). The smallest female to exhibit maturity (stage 4 or higher) was 157.5 cm with an estimated weight of 66 kg and contained extensive atresia of vitellogenic oocytes. According to Restrepo *et al*. (2010), this fish was 6 y old. Lipid stage oocytes are the first step in oocyte maturation and were present in most females, including those smaller than 185 cm, sampled during June–July in the Gulf of Maine foraging grounds (**Figure 2**). This could indicate a mature or maturing individual as Corriero *et al*. (2003) reported 70% of pre-spawning, mature size Mediterranean ABFT had lipid stage oocytes as the most advanced stage. The same study also showed all fish of immature size sampled between May and September (pre to early post season) had perinucleolar oocytes as the most advanced stage.

The FSH/LH ratio of ABFT between 134–185 cm CFL is also characteristic of mature ABFT. While FSH is the dominant gonadotropin in young fish, LH is dominant among mature individuals (**Figure 3**). This is also in agreement with analyses of immature and mature ABFT from the Mediterranean (Rosenfeld, unpublished data).

While sampling gonads on the spawning grounds offers a direct assessment of the reproduction status of spawning fish, the consequent conclusions should be limited to that spawning location. By combining endocrine and histological analyses for individuals located outside of known spawning grounds, we obtained insights on reproductive profiles across a large spatial and temporal range, which is more likely to reflect the entire stock. Our results do not support the suggested increase in age at maturity for ABFT (Diaz and Turner 2007, Diaz 2011), and our results showed no indications for postponed maturation in western ABFT as compared to Mediterranean ABFT (Corriero *et al*. 2005). In addition, results from condition studies, skipped spawning (Jørgensen *et al*. 2006, Rideout *et al*. 2005), and life history modeling (Chapman *et al*. 2011) do not support the hypothesis that a large number of sexually mature and energetically competent ABFT would skip spawning, except for the youngest, recently mature fish (Holland *et al*. 2001). It's possible that some of the smaller fish included in our sample were of eastern Atlantic origin, but based on the extensive spatio-temporal range of our sampling it's highly unlikely that all of them were, so combined results most likely represent the maturity profile of western Atlantic ABFT.

Our results suggest that further sampling in potential alternative western Atlantic spawning grounds (Lutcavage *et al*. 1999, Galuardi *et al*. 2010) is necessary in order to investigate the reproductive potential of western ABFT (Fromentin and Powers 2005; Takeuchi *et al*. 2008). Although we can predict potential spawning areas based on spatio-temporally explicit results from electronic tagging (Lutcavage *et al*., SCRS/x/2012) and oceanographic profiling (Teo *et al*. 2007, Galuardi *et al*. 2010), this has proven difficult (Lutcavage and Luckhurst 2001) as offshore spawning areas are hard to sample because of logistics and US regulatory restrictions on ABFT retention. In addition, since we suspect offshore longline fisheries only sporadically encounter spawning ABFT, and potential Atlantic spawning areas are likely to change, the evaluation of western spawning and larval development areas will require far more extensive sampling. Following Mather *et al* (1995): "Considering the amount of research which has been devoted to the Atlantic bluefin tuna, positive information on its spawning habits is surprising incomplete," a more comprehensive understanding of reproduction in western ABFT is now within reach and, so far, confirms historical findings on maturity status.

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Table 1. Biometric data are summarized for Atlantic bluefin tuna, *Thunnus thynnus*, sampled from the NW Atlantic between 2004 and 2011. YOY are not included here as they contained undifferentiated gonads. SFL=straight fork length; GW=gonad weight; GSI=gonadosomatic index.

Sex		CFL (*cm*)	GW (*g*)	GSI
Male	μ (±SD)	214 (±32.4)	303.1(±317.9)	0.17(±0.160)
	Range	112–292	4.0–2100	0.01–1.07
	n	304	319	311
Female	μ (±SD)	206 (±34.86)	602.1(±389.2)	0.39(±0.215)
	Range	107–292	3.0–2086	0.01–1.35
	n	206	210	206

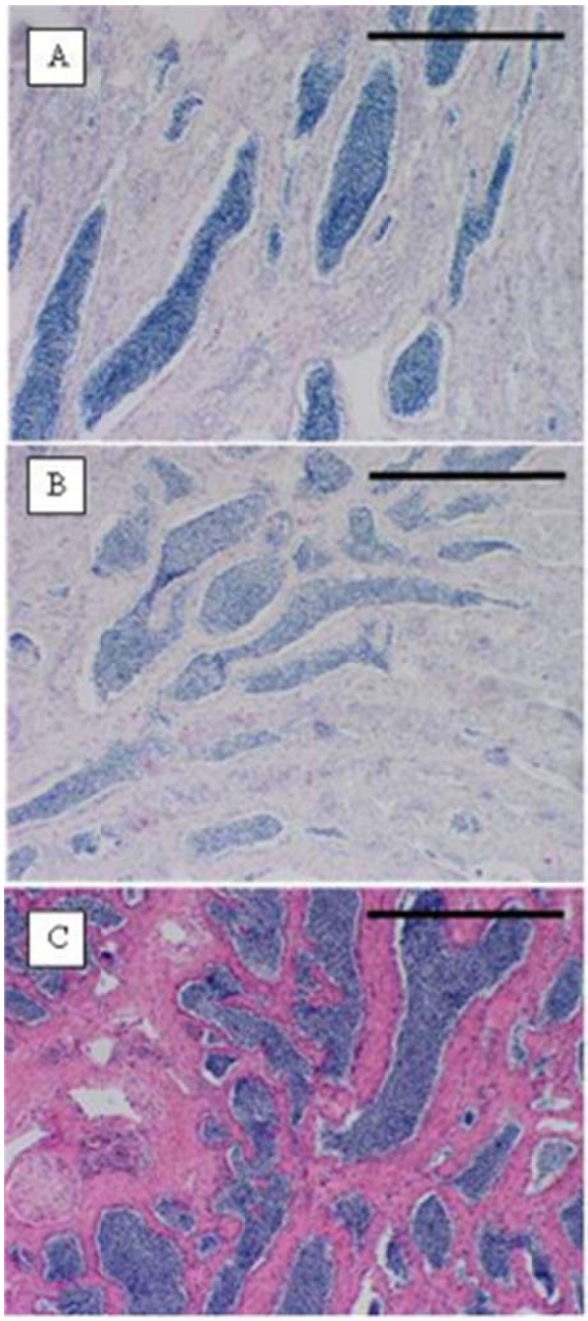

Figure 1. Partially spent testes of relatively small ABFT (145–169 cm) sampled during August in the Gulf of Maine, NW Atlantic (A) 145 cm male, estimated body weight of 51 kg, testis weight- 50 g, fat body weight- 150 g; (B) 145 cm male, estimated body weight of 51 kg, testis weight- 50 g, fat body weight- 100 g; (C) 169 cm male, estimated body weight of 81 kg, testis weight- 500 g, fat body weight- 300 g. Milt was present and flowing out of the testes when cut. All testes were in absorbed condition. Bar = 300 µm

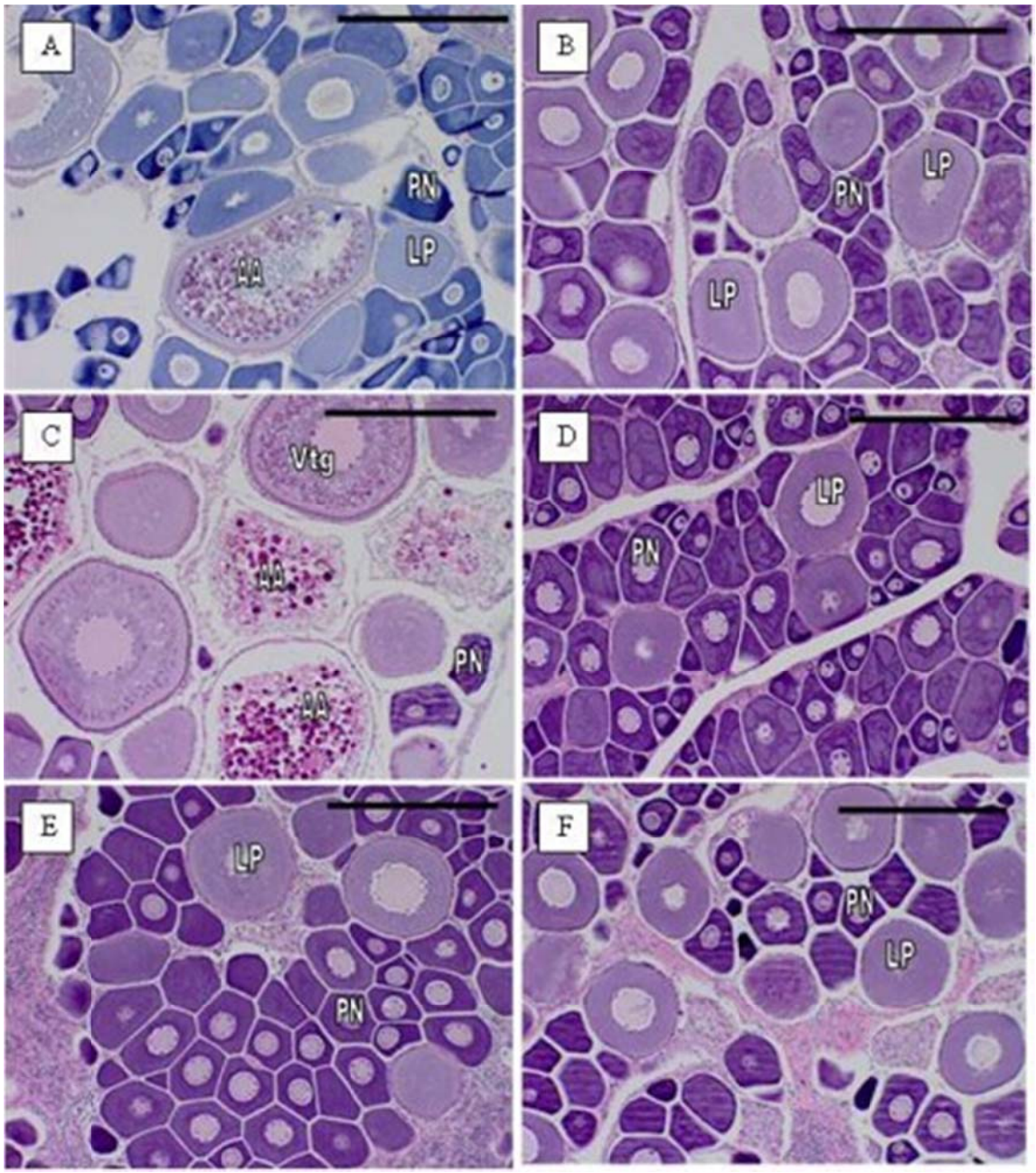

Figure 2. Micrographs of bluefin tuna ovaries sampled during early foraging season (June–July) in the Gulf of Maine, NW Atlantic. (A) 190 cm female exhibiting perinucleolar stage, lipid stage oocytes, as well as alpha atresia; (B) 198 cm female with lipid stage oocytes as the most advanced stage; (C) 208 cm female exhibiting perinucleolar, lipid and vetelogenic stage oocytes, as well as alpha atresia; (D–F), 162, 154 and 157 cm females, respectively, with lipid stage oocytes as the most advanced stage. PN - perinucleolar stage; LS - lipid stage; Vtg vetelogenic oocyte; AA - alpha atresia. Bar = 300 µm.

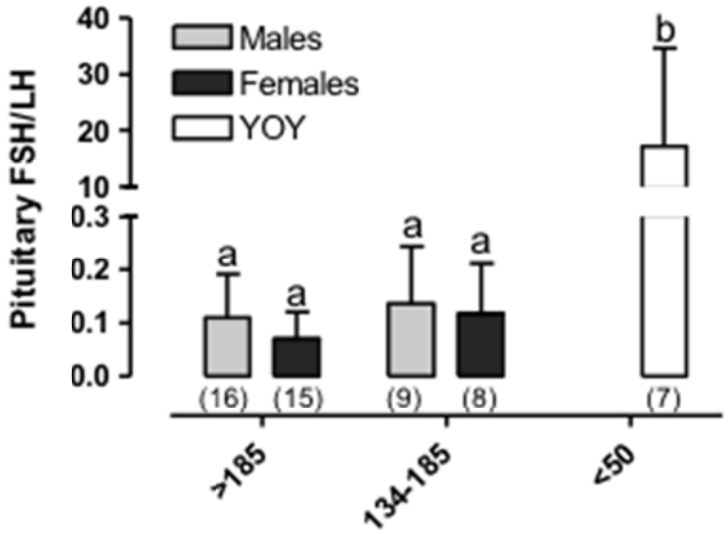

Figure 3. Average FSH/LH hormones ratio. Protein FSH levels (µg/pit/BW) divided by protein LH levels (µg/pit/BW) for each individual and then averaged for comparison. On the x-axis are the fish grouped by size (curved fork length in cm). All fish larger than 134 cm had FSH/LH protein ratios smaller than 0.36 (total average: 0.1 ± 0.08). All YOY ABFT (<50 cm) were sampled on September 13, 2008 off Virginia, NW Atlantic. FSH/LH ratio for YOYs ranged between 2.00 to 46.05. Different letters above error bars (SDV) indicate significant difference between means (Tukey-Kramer, α=0.05). Sample numbers are in parentheses.