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AGE AND GROWTH OF LARVAL ATLANTIC BLUEFIN TUNA, *THUNNUS THYNNUS*, FROM THE GULF OF MEXICO

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SUMMARY

*Atlantic bluefin tuna (*Thunnus thynnus*) are highly pelagic, undertaking extensive migrations throughout the Atlantic. They spawn primarily in the Mediterranean Sea and Gulf of Mexico. Despite 30 years of ichthyoplankton surveys in the Gulf of Mexico little is known about bluefin early life history and larval growth. In this study, we describe preliminary age-length relationships for larval Atlantic bluefin tuna using otolith microincrement analysis. Larvae were collected from plankton tows in the Gulf of Mexico in April-May 2012. Otoliths (sagittae) were dissected from 50 larvae, ranging from 2.4 to 7.4 mm (NL or SL) with ages from 4-15 days. From these data we developed new growth curves for the Gulf of Mexico. Growth was highly variable at a given length, which likely reflects environmental variability encountered in the dynamic oceanographic environment of the Gulf of Mexico. Results will improve the annual larval index, which currently uses an age-length relationship based on specimens collected solely off South Florida more than 30 years ago.*

RÉSUMÉ

*Le thon rouge de l'Atlantique (*Thunnus thynnus*) est un grand migrateur pélagique réalisant de longues migrations dans l'ensemble de l'Atlantique. Il fraie principalement en mer Méditerranée et dans le golfe du Mexique. En dépit de 30 ans d'études sur l'ichthyoplancton menées dans le golfe du Mexique, les connaissances sont lacunaires en ce qui concerne les premières étapes du cycle vital et la croissance larvaire. La présente étude décrit les relations préliminaires âge-taille des larves du thon rouge de l'Atlantique obtenues au moyen de l'analyse de micro-incrément des otolithes. Les larves ont été prélevées dans des traits planctoniques dans le golfe du Mexique en avril-mai 2012. Des otolithes (sagittae) ont été extraits par dissection de 50 larves, mesurant entre 2,4 et 7,4 mm (NL ou SL) et âgées de 4 à 15 jours. Sur la base de ces données, nous avons élaboré de nouvelles courbes de croissance pour le golfe du Mexique. La croissance était extrêmement variable à une taille donnée, ce qui s'explique vraisemblablement par la variabilité environnementale de l'environnement océanographique dynamique du golfe du Mexique. Les résultats amélioreront l'indice annuel larvaire, qui utilise actuellement une relation âge-taille fondée sur des spécimens prélevés uniquement au large du Sud de la Floride il y a plus de 30 ans.*

RESUMEN

*El atún rojo del Atlántico (*Thunnus thynnus*) es un gran migrador pelágico, que realiza amplias migraciones por todo el Atlántico. Desova principalmente en el Mediterráneo y en el golfo de México. A pesar de 30 años de prospecciones de ictioplancton en el golfo de México, se sabe poco acerca de las primeras etapas del ciclo vital del atún rojo y del crecimiento de las larvas. En este estudio se describen las relaciones edad-talla preliminares para las larvas de atún rojo del Atlántico utilizando análisis del microincremento de otolitos. Las larvas se recogieron de redes de plancton en el golfo de México en abril-mayo de 2012. Se diseccionaron los otolitos (sagittae) de 50 larvas, y oscilaban entre 2,4 a 7,4 mm (NL o SL), con edades entre 4 y 15 días. A partir de estos datos, se han desarrollado nuevas curvas de crecimiento para el golfo de México. El crecimiento era muy variable en una determinada talla, lo que*

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probablemente refleja la variabilidad medioambiental encontrada en el dinámico entorno oceanográfico del golfo de México. Los resultados mejorarán el índice anual de larvas, que actualmente utiliza una relación edad-talla basada en ejemplares recogidos únicamente en aguas del sur de Florida hace más de 30 años.

KEYWORDS

Age determination, Fish larvae, Growth curves, Otoliths, Life history, Atlantic bluefin tuna

1. Background

Bluefin tuna migrate to spawn in the Gulf of Mexico (western stock) and Mediterranean Sea (eastern stock) (Block *et al.*, 2005) over a limited time period: April to May in the Gulf of Mexico, and June to July in the western Mediterranean. Although it is understood that bluefin tuna undergo extensive migrations to warm, oligotrophic waters to deposit their larvae, little is known about the subsequent growth and mortality of these early life stages.

Average larval abundances of bluefin tuna are used to develop annual indices to support stock assessments (Scott *et al.*, 1993). This is the only fishery independent source of information that is currently applied to the western stock's population assessment (Ingram *et al.*, 2010). These indices utilize larval age at length derived from otoliths to back-calculate observed abundances to equivalent abundances of one day old larvae. However, despite 30 years of ichthyoplankton surveys, the age and growth curve used is based upon a geographically restricted set of samples collected off Miami in 1980-1981 (Brothers *et al.*, 1983). These samples represent a very small sub-sample of the total population, which were not sourced from the main spawning ground (the Gulf of Mexico). By restricting age and growth estimates to limited collections taken over one sampling period, the current larval index does not take into account interannual and geographical variability in larval growth.

Otoliths allow a historical analysis of the life history of fishes (Campana 1992). Daily increments form at a constant frequency that can be measured using image analysis (Campana and Jones 1992; Sponaugle 2009). Repeated measurements (increment width, otolith radius) of sufficient sample sizes can reveal patterns in otolith growth (fast vs slow) and reflect spatial and temporal variability in larval growth (Jenkins and Davis 1990, Wexler *et al.*, 2001).

In this study, we 1) estimated the daily age from otoliths of larval Atlantic bluefin tuna from the 2012 spawning season, 2) developed growth curves using larval length (mm) and otolith radius length (μm), 3) compared observed age at length results with those described by Brothers *et al.*, 1983.

This and future work will provide an updated growth curve for larval indices, and will compliment other ongoing efforts to examine feeding success and prey selectivity of larvae at different life stages. Additionally, it will advance climate change research aiming to determine the influence of changing environmental conditions on growth and survival.

2. Methods

The age of larval bluefin tuna (in days) can be determined directly by examining their otolith microstructure (Brothers *et al.*, 1983, Itoh *et al.*, 2000, Garcia *et al.*, 2013). Otoliths are easily viewed under high magnification (100x-1000x) and have a recognizable pattern seen as continuous bipartite daily increments (Secor 1995). Itoh *et al.*, (2000) validated daily increment formation in laboratory reared fish from day 5 to day 71 after fertilization.

Bluefin larvae were collected for age estimation in April and May 2012 during the Southeast Area Monitoring and Assessment Program (SEAMAP) spring plankton cruise (Fig. 1) using two 1 x 2 m plankton nets. The first net (S-10, 505μm mesh) was towed in an undulating manner in the upper 10 m of the water column. The second net (neuston, 947μm mesh) was towed at the air-sea interface for 10 minutes. Detailed sampling methodologies are described in Habtes *et al.*, 2014. Samples were preserved in 70-95% ethanol to conserve tissues and otolith structure.

Bluefin larvae were identified following SEAMAP protocols and identification keys (Richards 2006). Body length (SL, mm) was measured using a dissecting microscope (Leica M205C), which was equipped with a digital camera (Leica DFC290HD) and image analysis software (Image Pro Plus 7) previously calibrated with an ocular micrometer.

Sagittal otoliths were blind read twice by three readers using digital micrographs captured with an Olympus BH2 compound microscope (40x-1000x) with immersion oil using transmitted light. The Image Pro caliper tool was used to mark and measure the maximum otolith radius (OR), (Fig. 2) as the reading axis for all readers. The highest and lowest values of the six reads were excluded, and mean values were assigned per larval fish. Itoh *et al.*, (2000) and Brothers *et al.*, (1983) report age estimates for larval bluefin tuna by adding four days to the observed otolith increments. These four days correspond to one day allocated for hatching plus three days until the onset of exogenous feeding to the final increment count. Consequently observed reads from this study were converted to ages by adding 4 days to the mean increment count. The Coefficient of Variation (CV) was calculated to measure the precision of multiple reads for each larval fish (Chang, 1982).

$$CV = 100\% \times \frac{\sqrt{\sum_{i=1}^{R} \frac{(X_{ij} - X_j)^2}{R-1}}}{X_j}$$

Growth rates were calculated by dividing the length (SL, mm) with observed age (days). **Figures 3 and 4** from Brothers *et al.* (1983) were digitized and overlaid with the current study for comparison. Growth curves were fitted as least squares regression of length at age. Increment widths from this study were measured at the edge of each D-zone and the mean values were plotted with body length. Spawning dates were calculated by subtracting the age (days) from the collection dates.

3. Results

A total of 1,346 bluefin tuna larvae were identified from 120 stations sampled in Gulf of Mexico during the Spring SEAMAP 2012 cruise (**Figure 1**). Larvae were subsampled from the east and west of longitude 90° within the US Gulf of Mexico EEZ throughout the cruise period 30 April -27 May 2012.

Fifty bluefin tuna larvae were aged from 27 stations sampled throughout the GOM region. 46 larvae were collected using the S-10, and 4 came from neuston samples. All otoliths examined under transmitted light displayed a primordium encircled by one or two diffuse zones followed by daily increments composed of a transparent layer (continuous L-zones that appear white) and a darker often wider layer (discontinuous D-zones that appear dark) (Secor 1995), (**Figure 2**). Although some otoliths were difficult to read, no otoliths were excluded for ageing. Larval fish ranged in size from 2.43 to 7.42 mm SL (**Table 1**). Ages ranged from 4.75-14.50 days post-fertilization (**Figure 3**). Spawning dates for this data set started on 16 April 2012 and continued almost daily through 21 May 2012. The least squares regression in the linear form was fitted to the data resulting in: $y = mx + b$ (N=50, m=0.39, b=1.34), r^2=0.74. The mean CV was 4.2 % and ranged from 0-12.4%. These measures of precision are the first reported values for Atlantic bluefin tuna from the western Gulf of Mexico.

The otolith radius has a positive relationship with body length (Brothers *et al.*, 1983). The OR ranged from 11-55 µm (Fig. 4). The least squares regression in the exponential form was fitted to the observed otolith measurements resulting in: $y=ae^{bx}$ (N=50, a = 5.07, b = 0.32, r^2 = 0.89). Results fall within reported values for increment values by Itoh *et al.*, 2000 and Brothers *et al.*, 1983. The OR for the first increment was 11.38 ± 1.13 µm (mean ± SD) corresponding to a 5 day old larval fish.

Figures 3 and 4 from Brothers *et al.*, (1983) were digitized using Surfer 9 and overlaid with the current study for comparison (see **Figures 3 and 4**). Mean values for size (SL, mm) at age (days), OR (µm), and growth rates (mm day^{-1}) are reported and compared in **Table 1**.

Growth was highly variable for the larvae examined. The observed growth rate was 0.52 ± 0.6 mm day^{-1} (mean ± SD). These values are lower than Brothers *et al.*, (1983), however our dataset did not include fish larger than 7.4 mm SL. Otolith measurements showed that growth was relatively uniform for the first week (1-2 µm), then increasing quickly (>3 µm) but with higher variability as the fish aged (Fig. 5). This study shows a linear fit for age at length curves with similar parameters to the linear regressions published for larval bluefin tuna from the Mediterranean (Garcia *et al.*, 2013). Observed values have similar slopes indicating comparable growth for larvae spawned in the Gulf of Mexico for 2012 and the Mediterranean from 2004 and 2005. In future work, additional larvae will be aged from the same collection years to compare GOM larvae to Mediterranean spawned larvae.

4. Conclusions

Larval growth and mortality studies on larval bluefin tuna have not been published for the Gulf of Mexico. The ecology of larvae spawned into this environment is essentially unknown. In 2012, larvae were distributed throughout the Gulf of Mexico, however, collections were patchy. The mean observed age was 11.22 ±2.4 days and the majority of fish aged (90%) were older than 8 days old. The 1981 collections from Brothers *et al.*, (1983) included a larger size range of larvae; however our results indicate slower growth than Brothers *et al.* (1983). Calculated spawning dates from this study were almost continuous possibly reflecting clumped but frequent spawning during the spawning season for the western stock of bluefin tuna.

Although sampling took place in May for this study and for Brothers *et al.*, (1983), a direct comparison is difficult given the disparity in sampling durations and geographic coverage. The larvae aged in Brothers *et al.*, (1983) were collected at the inshore edge of the Gulf Stream (**Figure 1**) as it passes Miami on two separate sampling efforts in May (Richards, pers. com, Brothers *et al.*, 1983). These larvae represent a small subset of bluefin in the Gulf of Mexico and would not reflect the wide range of temperatures and feeding conditions that BFT larvae may be exposed to in the rest of their spawning grounds. For instance, SST ranged from 21° to 29°C during the sampling period for the 2012 survey, and many of the larvae may be entrained in cyclonic or anticyclonic eddies with differing temperatures and productivity. The SEAMAP cruise covers the Gulf of Mexico during the majority of the bluefin tuna spawning season each spring. This maximizes opportunities to collect larvae exposed to a variety of environmental conditions which will likely be reflected in otolith microstructure. Future efforts will focus on determining growth rates on a weekly basis during the spawning season in the Gulf of Mexico. In addition, we will investigate the contribution of mesoscale oceanographic features to variability in growth rates.

Recent research indicates that growth and survival of bluefin tuna larvae is highly variable, both inter-annually and spatially (Garcia *et al.*, 2013). Upon completion of this work, additional otoliths will be aged to include a wider range of sizes, collection locations and years. In addition, further ageing of available larvae can determine growth and mortality rates in different environments, and larval transport through oceanographic features to greatly improve our understanding of yearly fluctuations in growth. These data are critical to improving our understanding of factors affecting recruitment, which are essential for parameterization of stock assessment models, and thus effective management.

References

Block, B.A., Teo, S.L.H., Walli, A., Boustany, A., Stokesbury, M.J.W., Farwell, C.J., Weng, K.C., Dewar, H., Williams, T.D. 2005. Electronic tagging and population structure of Atlantic bluefin tuna. Nature 434: 1121-1127.

Brothers, E. B., Prince, E. D. Lee, D. W. 1983. Age and growth of young-of-the-year blue fin tuna, *Thunnus thynnus,* from otolith microstructure. Pages 49-60 *in* E. D. Prince and L. M. Pulos, eds. Proceedings of the international workshop on age determination of oceanic pelagic fishes: tunas, bill fishes, and sharks. NOAA Tech. Rep. NMFS 8. 211 pp.

Campana, S.E. 1992. Measurement and interpretation of the microstructure of fish otoliths, p. 59-71. D.K. Stevenson and S.E. Campana [ed.]. Otolith microstructure examination and analysis. Can. Spec. Publ. Fish. Aquat. Sci. 117.

Campana S.E. and Jones C.M. 1992. Analysis of otolith microstructure data, p. 73-100. D.K. Stevenson and S.E. Campana [ed.]. Otolith microstructure examination and analysis. Can. Spec. Publ. Fish. Aquat. Sci. 117.

Chang, W.Y.B. 1982. A statistical method for evaluating the reproducibility of age determination. Can. J. Fish. Aquat. Sci. 39:1208-1210.

García, A., Cortés, D., Quintanilla, J., Rámirez, T., Quintanilla, L., Rodríguez, J. M., & Alemany, F. 2013. Climate-induced environmental conditions influencing interannual variability of Mediterranean bluefin (*Thunnus thynnus*) larval growth. Fish. Oceanogr. 22:4,273-287.

Habtes, S., Muller-Karger, F. E., Roffer, M. A., Lamkin, J. T., & Muhling, B. A. 2014. A comparison of sampling methods for larvae of medium and large epipelagic fish species during spring SEAMAP ichthyoplankton surveys in the Gulf of Mexico. Limnology and Oceanography - Methods 12: 86-101.

Ingram, G.W., Richards, W.J., Lamkin, J.T., Muhling, B.A. 2010 Annual indices of Atlantic bluefin tuna (*Thunnus thynnus*) larvae in the Gulf of Mexico developed using delta-lognormal and multivariate models. Aquatic Living Resources 23: 35-47.

Jenkins, G.P. and Davis, T.L.O. 1990. Age, growth rate, and growth trajectory determined from otolith microstructure of southern bluefin tuna *Thunnus maccoyii* larve. Mar. Ecol. Prog. Ser. 63:93-104.

Itoh, T., Shiina, Y., Tsuji, S., Endo, F., & Tezuka, N. 2000. Otolith daily increment formation in laboratory reared larval and juvenile bluefin tuna *Thunnus thynnus*. Fisheries Science, 66(5), 834-839.

Scott, G.P., Turner, S.C., Grimes, C.B., Richards, W.J., Brothers, E.B. 1993. Indices of larval bluefin tuna, *Thunnus thynnus*, abundance in the Gulf of Mexico: Modeling variability in growth, mortality, and gear selectivity: Ichthyoplankton methods for estimating fish biomass. Bulletin of Marine Science 53: 912-929.

Secor, D. H., Dean, J. M., & Campana, S. E. 1995. Recent developments in fish otolith research. University of South Carolina Press, Colombia.

Sponaugle, S. (2009). Daily otolith increments in the early stages of tropical fishes. Pages 93-132 *In*: B. Green, B. Mapstone, G. Carlos, and G. Begg, eds. Gathering information from otoliths of tropical fishes. Methods and Technologies in Fish Biology and Fisheries. Springer.

Wexler, J.B., Margulies, D., Masuma, S., Tezuka, N., Teruya, K., Oka, M., Kanematsu, M, and Nikaido, H. 2001. Age validation and growth of yellofin tuna *Thunnus albacares*, larvae reared in the laboratory. Inter-Am. Trop. Tuna Comm. Bull. 22:52-91

Table 1. Mean values for body length (mm), otolith radius (µm) and growth rates (mm/day) from this study and from Brothers *et al.*, (1983).

	Length (SL mm)			*Otolith radius, µm*			*Growth Rate (mm/day)*		
	Mean	*Min*	*Max*	*Mean*	*Min*	*Max*	*Mean*	*Min*	*Max*
Brothers *et al.* 1983, n=328	5.10	3.81	8.91	28.82	19.0	88.0	0.69	0.52	1.38
This study , n=50	5.71	2.43	7.42	34.01	11.05	55.23	0.52	0.40	0.69

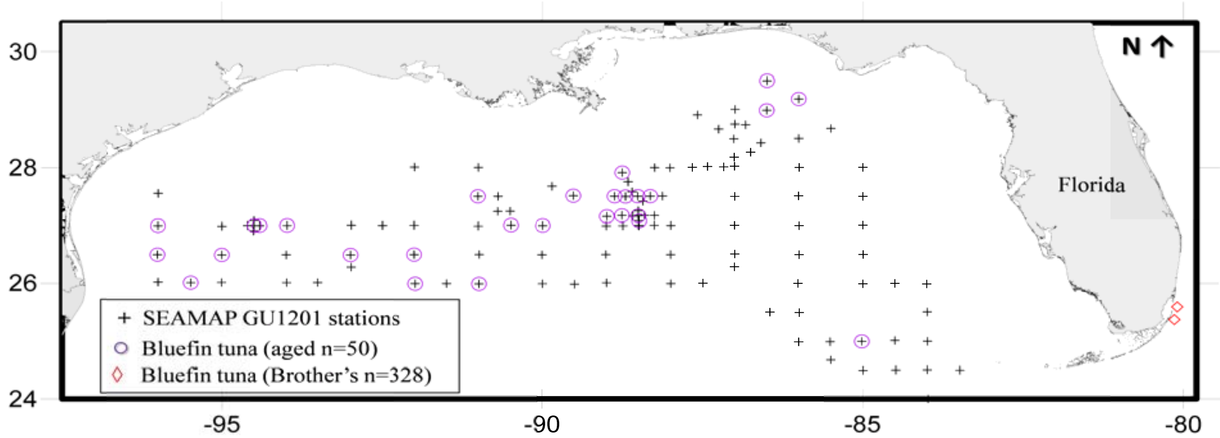

Figure 1. Stations + (n=129) sampled during SEAMAP cruise GU1201 (29 April – 29 May 2012) in the Gulf of Mexico. Open circles ○ (n=29) shows station location of aged *Thunnus thynnus*, diamonds (◊) indicate stations sampled in Brothers *et al.* (1983).

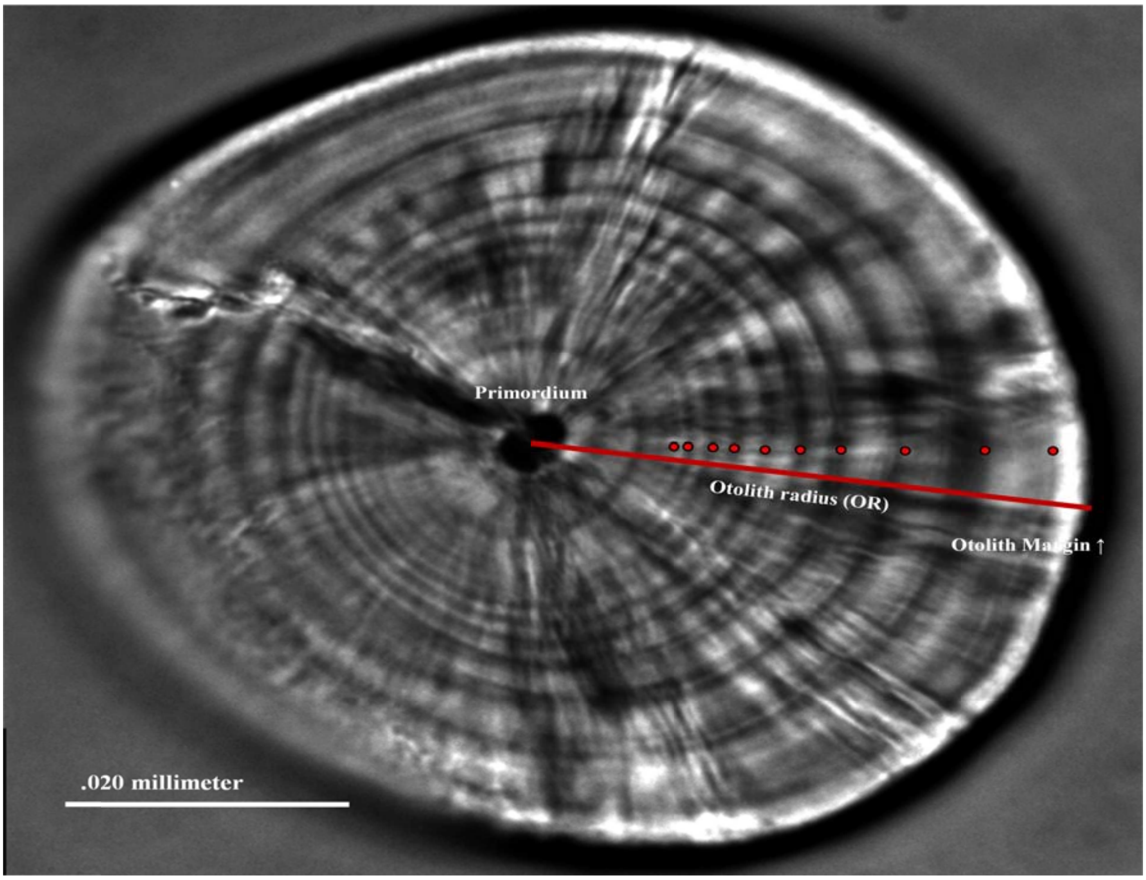

Figure 2. Microstructure of larval bluefin tuna otolith at 1000x. Distal surface of sagittal otolith from a 6.6 mm SL larva collected from the NW Gulf of Mexico on May 21, 2012. The red line indicates the otolith radius (the maximum distance from primordium to the otolith margin). Inside the primordium, diffuse zones are seen. Daily increments are shown closed circles (●) at the end of each discontinuous zone. Scale indicates 0.02 mm.

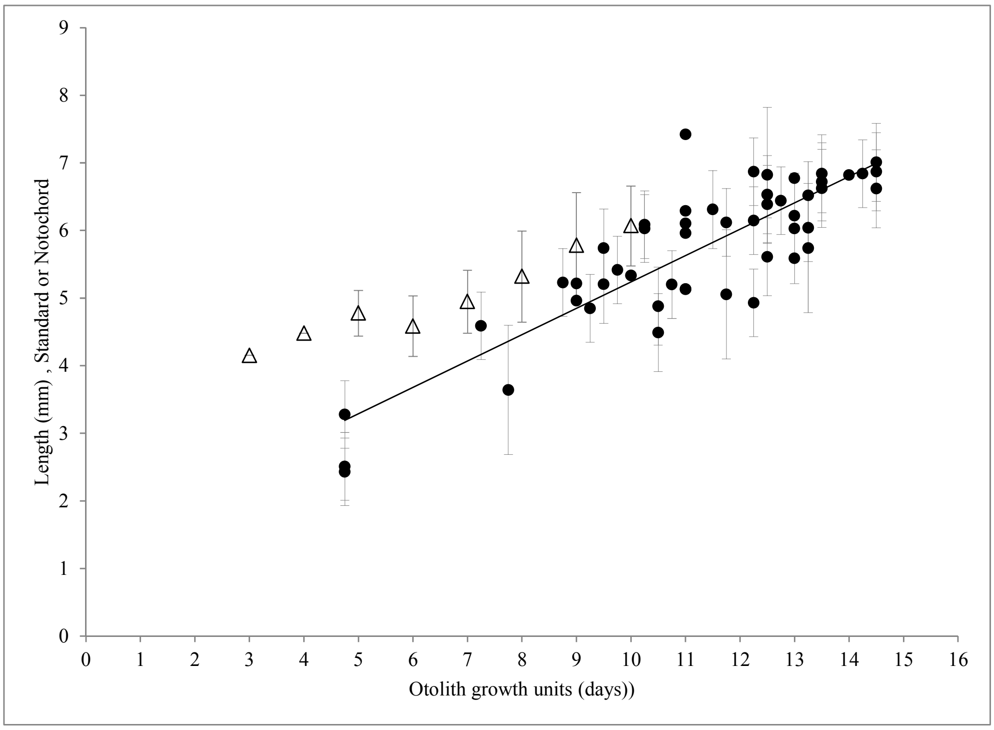

Figure 3. Otolith age (days) and fish length (SL or NL) for *Thunnus thynnus*. Closed circles (●) show otolith growth units (days) vs bluefin tuna length (SL or NL, mm). Least squares regression in the linear form is $y = mx + b$ (N=50, m=0.39, b=1.34), r^2=0.74. Open symbol (Δ) indicate the mean length at age from Figure 3 of Brothers *et al.*, (1983), N=328. Error bars denote standard deviation.

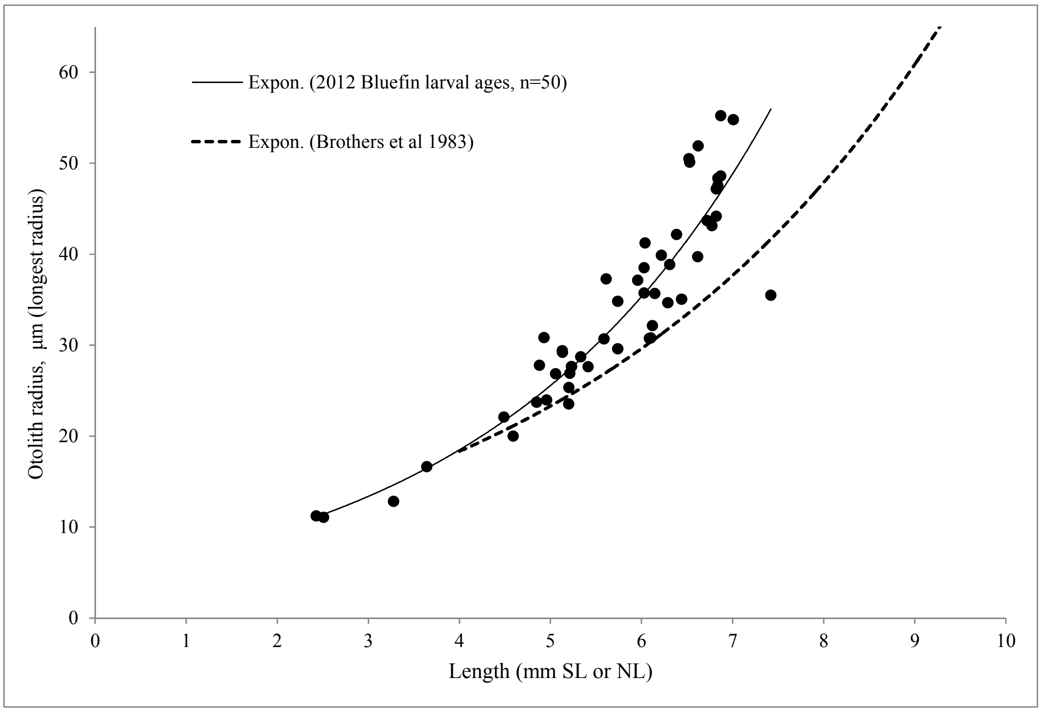

Figure 4. Scatter plot of *Thunnus thynnus* length (SL or NL) mm and otolith radius (µm). The least squares regression exponential form y=aebx (N=50, a = 5.07, b = 0.32, r2 = 0.89). Dotted line denotes Brothers *et al.* 1983 relationship with the same least squares regression $y=ae^{bx}$ (N = 90, a = 7.02, b = 0.24, r^2 = 0.71).

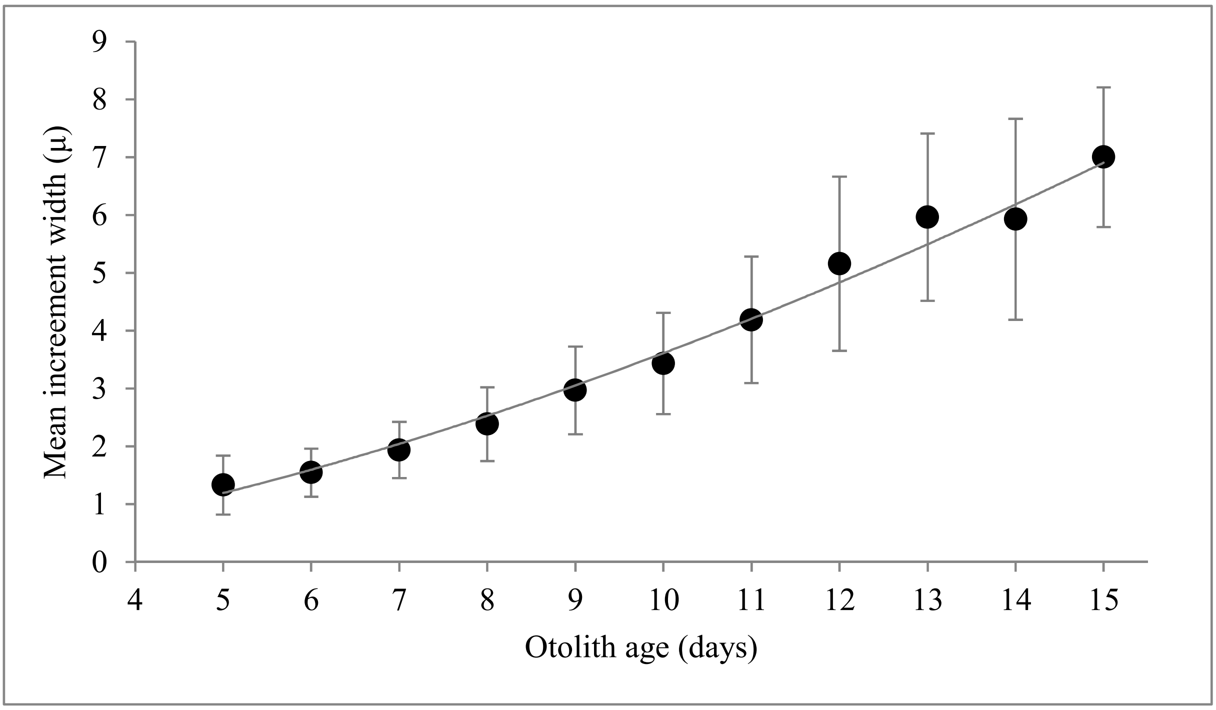

Figure 5. Otolith age (days) at observed mean increment widths (µm) for n=50 sagittal otoliths. Error bars denote standard deviation. Line indicates the least squares regression power function $y = ax^b$, a=0.0912, b=1.58, $r^2 = 0.99$.