A possible new spawning area for bluefin tuna (*Thunnus thynnus*): First histologic evidence of reproductive activity in southern Gulf of Mexico

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The number of studies on reproductive biology carried out in the Gulf of Mexico is lower than those undertaken in the Mediterranean Sea. Four spawning areas have been found for the eastern Atlantic bluefin tuna stock in the Mediterranean Sea. So it is not implausible that there is more than one spawning area in the Gulf of Mexico for the western Atlantic bluefin tuna stock. The bluefin tuna used in this work were all caught by the Mexican surface longline fleet between January and April 2015. A total of 63 individuals ranging between 192 and 293 cm L_F (mean = 238 ± 22.52 cm) were measured. Gonads from 46 fish (31 females and 15 males) were collected for histological examination. All the individuals were classified as mature; 25 were reproductively active (in spawning capable and spawning stages). The histological analysis indicates spawning activity in Mexican waters. Spawning occurred in March and April, when the sea surface temperature was 25.5°C ± 0.687 in March and 27°C ± 0.685 in April.

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Abstract

The number of studies on reproductive biology carried out in the Gulf of Mexico is lower than those undertaken in the Mediterranean Sea. Four spawning areas have been found for the eastern Atlantic bluefin tuna stock in the Mediterranean Sea. So it is not implausible that there is more than one spawning area in the Gulf of Mexico for the western Atlantic bluefin tuna stock. The bluefin tuna used in this work were all caught by the Mexican surface longline fleet between January and April 2015. A total of 63 individuals ranging between 192 and 293 cm L_F (mean = 238 ± 22.52 cm) were measured. Gonads from 46 fish (31 females and 15 males) were collected for histological examination. All the individuals were classified as mature; 25 were reproductively active (in spawning capable and spawning stages). The histological analysis indicates spawning activity in Mexican waters. Spawning occurred in March and April, when the sea surface temperature was 25.5°C ± 0.687 in March and 27°C ± 0.685 in April.

Introduction

Atlantic bluefin tuna, *Thunnus thynnus* (Linnaeus 1758) is a large, highly migratory species distributed in the Atlantic Ocean between 70°N and 30°S latitudes (Collette and Nauen 1983). Two different bluefin tuna stocks are considered for management purposes, the eastern and the western Atlantic stocks, separated at the 45°W meridian. This borderline was based on the two well-known spawning areas, the Mediterranean Sea and Gulf of Mexico. Atlantic bluefin tuna is

classified as an endangered species by the International Union for Conservation of Nature (IUCN) red list as a consequence of fishing pressure (Collette et al. 2011*a*,*b*). Currently, there are ICCAT regulations aimed at managing both stocks (https://www.iccat.int/en/RecRes.asp). Although the western stock was the first to be under regulation (since 1999), the number of reproductive studies is lower than those undertaken for the eastern stock (Susca et al. 2001; Corriero et al. 2003; Aranda et al. 2011; MacKenzie and Mariani, 2012).
The studies of the western stock focus on Canadian waters and the northern region of the Gulf of Mexico, specifically in United States waters (Heinisch et al. 2014; Knapp et al. 2014). The spawning area was located in the north and northwest of the Gulf of Mexico (Nemerson et al. 2000; Ingram et al. 2010). Over the decades it has been thought that the western Atlantic bluefin tuna spawned only in the north Gulf of Mexico, but a recent larval study has reported a new spawning ground in the Slope Sea (Richardson et al. 2016). There remains a lack of information on the reproductive status of individuals at the time of their appearance in Mexican waters (Abad-Uribarren et al. 2014). In the Mediterranean Sea four spawning zones have been described (Karakulak et al. 2004). So it is quite plausible that more than one spawning area in the Gulf of Mexico can exist. The main objective of this work was to determinate the reproductive status of Atlantic bluefin tuna caught in Mexican waters (southern Gulf of Mexico) using histological examination of the gonads. Additionally, the size frequency of this species in the area was analyzed.

Materials & Methods

The individuals used in the present work were caught by the Mexican surface longline fleet targeting yellowfin tuna *Thunnus albacares* (Bonnaterre, 1788) from January to April 2015. Bluefin tuna individuals were measured to the nearest cm (fork length; L_F) by scientific observers on longline vessels. Length analysis was conducted using kernel density estimators (KDE) (Salgado-Ugarte 2002; Rivera-Velázquez et al. 2010). KDE equation is

$$\hat{f}(x) = \frac{1}{nh}\sum_{i=1}^{n} K\left(\frac{x - X_i}{h}\right)$$

Where $\hat{f}(x)$ is the density estimation of the variable x, *n* is the number of observations, *h* is the bandwidth, X_i corresponds to length of the *i-th* fish specimen and *K* is a smooth, symmetric kernel function integrating to one.
The location of each bluefin tuna was recorded (Fig. 1) and a subset of bluefin tuna was sampled to collect gonad tissues. Gonad samples were fixed in Bouin's liquid for 4 hours and subsequently preserved in 70% ethanol. For histological examination, a preserved gonad subsample was embedded in paraffin. Sections of 10 µm were cut and stained, using the Mallory's trichrome stain. Microscopic classification used for Atlantic bluefin tuna gonads was based on a modification of the criteria of Schaefer (1998) and Farley et al. (2013).

Six developmental oocyte stages were considered in this study: primary growth (PG), lipid-stage (LS), early vitellogenic (E-Vit), advanced vitellogenic (A-Vit), migratory nucleus (MG) and hydrated (HY) oocytes. The most advanced group of oocytes (MAGO) present within each ovary and, the presence/absence of: postovulatory follicles (POFs), atretic follicles and late stages of atresia were used to determinate the sexual maturity. Females were classified as mature if ovaries contained vitellogenic, MG or HY oocytes and/or atresia. Only mature females were found in the present study; ovaries were classified into the following five ovarian stages. Developing, ovaries contained E-Vit oocytes as MAGO, some atresia of vitellogenic oocytes may be present, and no POFs; spawning capable, when ovaries contained A-Vit oocytes, some atresia of vitellogenic oocytes may be present, and no POFs; spawning, POFs and /or oocytes in the latter two advanced developmental stages (MG and HY oocytes) are present and some atresia may be present, but only in limited amount. Regressing, when ovaries had LS or vitellogenic oocytes as MAGO, but abundant atretic follicles and no POFs. Finally, regenerating stage if ovaries contained PG or LS oocytes as MAGO, late stages of atresia and no POFs.

For males, four cellular stages, namely spermatogonia (SG), spermatocytes (SC), spermatids (SD), and spermatozoa (SZ), were microscopically differentiated and recorded. Testes stages were then assigned based on: the relative abundance of cysts containing the four cellular stages, the presence or absence of spermatozoa within seminiferous tubules, and the amount of sperm (when present) within the central longitudinal sperm duct (vas deferens). No immature male was found, and four stages for mature testes were defined: developing (early spermatogenesis), cysts with SG, some SC and SD and few SZ can be present; spawning capable (late spermatogenesis) cysts with abundant SD and some SZ within seminiferous tubules; spawning, some SD, plenty of SZ and sperm duct full of sperm; and regressing (spent), residual SZ.

Monthly variation of sea surface temperature (SST) data for the area of operation of the Mexican longline fleet were obtained from the Giovanni web database, which is a web-based application developed by Goddard Earth Sciences Data and Information Services Center (GES DISC) (http://Giovanni.sci.gsfc.nasa.gov/giovanni/) to correlate the mean SST with the different reproductive stages.

Results

A total of 63 individuals were caught as bycatch, 5 in January (7.93%), 8 in February (12.69%), 42 in March (66.66%) and 8 in April (12.69%). Sizes of these individuals ranged from 192 to 293 cm L_F, with a mean of 238 ± 22.52 cm L_F. The size structure was determined by a dominant mode of 235 cm L_F (Fig. 2). Gonads of 46 bluefin tuna specimens, 31 ovaries and 15 testes, were histologically examined to determinate their maturity stages (Tables 1 and 2). The five ovarian stages are described in Fig. 3. All ovaries collected in January and February were in regenerating stage. In March ovaries in the regenerating stage were also observed (29%), a few (5%) were in developing, whereas those in the spawning capable stage were found to be more frequent (57%) and 10% were in regressing stage. In April, 25% were developing, 50% were in spawning capable and 25% were in spawning. The four testes stages are described in Fig. 4. Only one male was collected in January, being in early spermatogenesis. No testes were collected in February. In March, 10% were early spermatogenesis, 50% were in late spermatogenesis, 30% were in spawning, and 10% were in regressing. In April, 25% were in late spermatogenesis, 25% were in spawning and 50% were in regressing.

Sea surface temperature in the southern Gulf of Mexico was increasing slightly from January to February, from 23.04 °C ± 0.692 to 23.42 °C ± 0.691, in March a temperature of 25.57 °C ± 0.687 was registered and finally in April the SST reached 27.03 °C ± 0.685 (Fig. 5).

Discussion

This is the first study that reports histological information for reproductive status of bluefin tuna in the southern Gulf of Mexico. The timing of the catches for this species (7.93% of catches in January, 12.69% in February, increasing substantially to 66.66% in March, and decreasing in April to 12.69%) suggests that bluefin tuna gradually arrive to Mexican waters in January and February, registering the highest catch in March perhaps due to increased feeding behavior before the spawning season and a decrease in April when spawning begins. A similar behavior has been previously described for *Thunnus orientalis* by Chen et al. (2006) who found a decrease in feeding when the spawning period starts, as well as for other tuna species (Rivas 1954).

It is known that the reproductive season of tunas is strongly linked with temperature and 24 °C is ideal for spawning (Schaefer 1998). The SST registered in the fishery zone is 25.5°C ± 0.687 in March and 27°C ± 0.685 in April, in agreement with SST in March and April reported in the northern spawning zone of bluefin tuna in the Gulf of Mexico, where larvae were found from 25°C to 28°C (Muhling et al. 2010).

Several studies indicate that the spawning period of bluefin tuna is about 3 months, from May to July for eastern stock and from April to June for western stock (Clay 1991; Knapp et al. 2014). According to Diaz and Turner (2007) the sizes of the individuals caught in this study from January to April correspond to sexually mature individuals. The histological examination of gonads indicates that 48% of female and 67% of males are reproductively active. Male individuals in spawning stage were found after mid-March and males in regressing stage with evidence of residual SZ from previous spawning were found in late March and early April. For female, the most frequent ovary stage in March was spawning capable and one female in spawning stage was found in April, corresponding with the spawning season for western stock (Schaefer 2001; Teo et al. 2007). From histological perspective, Mexican waters could be considered a new spawning area for bluefin tuna. Lutcavage et al. (1999) expressed the need to consider other possible spawning areas for western stock and discuss the possibility of a spawning area in the mid-Atlantic region, with similar hydrographic characteristics to the spawning area of the north Gulf of Mexico, and where sexually mature individuals stay from February to July. However they could not prove the existence of a new spawning zone due to the lack of histological evidence.

Conclusions

In the present study four facts provide evidences for considering the southern area of the Gulf of Mexico as a new possible spawning area: 1) There is a marked seasonal occurrence of individuals, 2) SST is appropriate to carry out the reproduction of this species, 3) the sizes correspond to sexually mature individuals and 4) we have found histological evidences of spawning. Additional studies are needed to support the southern Gulf of Mexico as a habitual spawning area.

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References

Abad-Uribarren, A., Meiners, C., Ramírez-López, K., and Ortega-Gárcia, S. 2014. Dinámica temporal de la captura incidental de atún aleta azul (*Thunnus thynnus*) y su relación con la variabilidad ambiental en aguas Mexicanas del Golfo de México. Col. Vol. Sci. Pap. ICCAT. **70**(2): 684-698.

Aranda, G., Aragón L., Corriero, A., Mylonas, C.C., De la Gándara, F., Belmonte, A., and Medina, A. 2011. GnRHa-induced spawning in cage-reared Atlantic bluefin tuna: An evaluation using stereological quantification of ovarian post-ovulatory follicles. Aquaculture. **317**: 255-259. doi:10.1016/j.aquaculture.2011.04.030.

Chen, K.S., Crone, P., and HSU, C.C. 2006. Reproductive biology of female Pacific bluefin tuna *Thunnus orientalis* from south-western North Pacific Ocean. Fisheries. Sci. **72**(5): 985-994. doi:10.1111/j.1444-2906.2006.01247.x.

Clay, D. 1991. Atlantic bluefin tuna (*Thunnus thynnus thynnus* (L.)): A review. Inter-Am. Trop. Tuna Comm. Spec. Rep. **7**: 89-180.

Collette, B.B., and Nauen, C.E. 1983. FAO species catalogue. Scombrids of the world. An annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. FAO. Fish.Synop. pp. 90-92.

Collette, B.B., Carpenter, K.E., Polidoro, B.A., Juan-Jordá, M. J., Boustany, A., Die, D. J., Elfes, C., Fox, W., Graves, J., Harrison, L.R., McManus, R., Minte-Vera, C.V., Nelson, R., Restrepo, V., Schratwieser, J., Sun, C.L., Amorim, A., Brick-Peres, M., Canales, C., Cardenas, G., Chang, S.K., Chiang, W.C., De Oliveira-Leite Jr, N., Harwell, H., Lessa, R., Fredou, F.L., Oxenford, H.A., Serra, R., Shao, K.T., Sumaila, R., Wang, S.P., Watson, R., and Yáñez, E. 2011*a*. High value and long life—double jeopardy for tunas and billfishes. Science. **333**(6040): 291-292. doi:10.1126/science.1208730.

Collette, B., Amorim, A.F., Boustany, A., Carpenter, K.E., de Oliveira Leite Jr., N., Di Natale, A., Die, D., Fox, W., Fredou, F.L., Graves, J., Viera Hazin, F.H., Hinton, M., Juan Jorda, M., Kada, O., Minte Vera, C., Miyabe, N., Nelson, R., Oxenford, H., Pollard, D., Restrepo, V., Schratwieser, J., Teixeira Lessa, R.P., Pires Ferreira Travassos, P.E. & Uozumi, Y. 2011*b*. *Thunnus thynnus*. *The IUCN Red List of Threatened Species* 2011: e.T21860A9331546. Available at http://dx.doi.org/10.2305/IUCN.UK.2011-2.RLTS.T21860A9331546.en (Downloaded on 30 October 2018).

Corriero, A., Desantis, S., Deflorio, M., Acone, F., Bridges, C.R., De la Serna, J.M., Megalofonou, P., and De Metrio, G. 2003. Histological investigation on the ovarian cycle of the bluefin tuna in the western and central Mediterranean. J. Fish. Biol. **63**(1): 108-119. doi:10.1046/j.1095-8649.2003.00132.x.

Diaz, G.A., and Turner, S.C. 2007. Size frequency distribution analysis, age composition, and maturity of western Bluefin tuna in the Gulf of Mexico from the U.S. (1981–2005) and Japanese (1975–1981) longline fleets. Col. Vol. Sci. Pap. ICCAT, **60**(4): 1160-1170.

Farley J.H., Williams A.J., Hoyle S.D., Davies C.R., and Nicol S.J., 2013. Reproductive dynamics and potential annual fecundity of South Pacific albacore tuna (*Thunnus alalunga*). *PloS ONE* **8**(4): e60577. doi:10.1371/jounal.pone.0060577.

Heinisch, G., Rosenfeld, H., Knapp, J.M., Gordin, H., and Lutcavage, M.E. 2014. Sexual maturity in western Atlantic bluefin tuna. Sci. Rep. **4**, 7205; doi:10.1038/ srep07205.

Ingram Jr, G.W., Richards, W.J., Lamkin, J.T., and Muhling, B. 2010. Annual indices of Atlantic bluefin tuna (*Thunnus thynnus*) larvae in the Gulf of Mexico developed using delta-lognormal and multivariate models. Aquat. Living. Resour. **23**: 35-47. doi:10.1051/alr/2009053.

Karakulak, S., Oray, I., Corriero, A., Deflorio, M., Santamaria, N., Desantis, S., and De Metrio, G. 2004. Evidence of a spawning area for the bluefin tuna (*Thunnus thynnus* L.) in the eastern Mediterranean. J. Appl. Ichthyol. **20**(4): 318-320. doi:10.1111/j.1439-0426.2004.00561.x.

Knapp, J.M., Aranda, G., Medina, A., and Lutcavage, M. 2014. Comparative Assessment of the Reproductive Status of Female Atlantic Bluefin Tuna from the Gulf of Mexico and the Mediterranean Sea. PLoS ONE **9**(6): e98233. doi:10.1371/journal.pone.0098233.

Lutcavage, M.E., Brill, R.W., Skomal, G.B., Chase, B.C., and Howey, P.W. 1999. Results of pop-up satellite tagging of spawning size class fish in the Gulf of Maine: do North Atlantic bluefin tuna spawn in the mid-Atlantic?. Can. J. Fish. Aquat. Sci. **56**(2): 173-177. doi:10.1139/f99-016.

MacKenzie, B.R., and Mariani, P. 2012. Spawning of Bluefin Tuna in the Black Sea: Historical Evidence, Environmental Constraints and Population Plasticity. PLoS ONE **7**(7): e39998. doi:10.1371/journal.pone.0039998.

Muhling, B.A., Lamkin, J.T., and Roffer, M.A. 2010. Predicting the ocurrence of Atlantic bluefin tuna (*Thunnus thynnus*) larvae in the northern Gulf of Mexico: Building a classification model from archival data. Fish. Oceanogr. **19**(6): 526-539. doi:10.1111/j.1365-2419.2010.00562.x.

Nemerson, D., Berkeley, S., and Safina, C. 2000. Spawning site fidelity in Atlantic bluefin tuna, *Thunnus thynnus*: the use of size-frequency analysis to test for the presence of migrant east Atlantic bluefin tuna on Gulf of Mexico spawning grounds. Fish. Bull. **98**(1): 118-126.

Richardson, D.E., Marancik, K.E., Guyon, J.R., Lutcavage, M.E., Galuardi, B., Lam, C.H., Walsh, H.J., Wildes, S., Yates, D.A., and Hare, J.A. 2016. Discovery of a spawning ground reveals diverse migration strategies in Atlantic bluefin tuna (*Thunnus thynnus*). PNAS. **113**(12): 3299-3304. doi:10.1073/pnas.1525636113.

Rivas, L.R. 1954. A preliminary report on the spawning of the western North Atlantic bluefin tuna (*Thunnus thynnus*) in the Straits of Florida. Bull. Mar. Sci. Gulf. Caribb. **4**(4): 302-322.

Rivera-Velázquez, G., Salgado-Ugarte, I., Soto, L., and Naranjo, E. 2010. Un estudio de caso en el análisis de la distribución de frecuencias de tallas de *Litopenaeus vannamei* (Boone, 1931) mediante el uso de estimadores de densidad por Kernel. Lat. Am. J. Aquat. Res. **38**(2): 201-209. doi:10.3856/vol38-issue2-fulltext-4.

Salgado-Ugarte, I.H. 2002. Suavización no paramétrica para análisis de datos. FES Zaragoza-DGAPA UNAM (PAPIIT IN217596, PAPIME 192031).

Schaefer, K.M. 1998. Reproductive biology of yellowfin tuna (*Thunnus albacares*) in the eastern Pacific Ocean. Inter-Am. Trop. Tuna Comm. Bull. **21**(5): 205-221.

Schaefer, K.M. 2001. Reproductive biology of tunas. *In* Tunas: physiology, ecology, and evolution. *Edited by* B.A. Block and E.D. Stevens. Academic Press, San Diego. pp. 225-270.

Susca, V., Corriero, A., Deflorio, M., Bridges, C.R., and De metrio, G. 2001. New results on the reproductive biology of bluefin tuna (*Thunnus thynnus*) in the Mediterranean. Col. Vol. Sci. Pap. ICCAT. **52**: 745-751.

Teo, S.L.H., Boustany, A.M., and Block, B.A. 2007. Oceanographic preferences of Atlantic bluefin tuna, *Thunnus thynnus*, on their Gulf of Mexico breeding grounds. Mar. Biol. **152**(5): 1105-1119. doi:10.1007/s00227-007-0758-1.

Figure 1

Fishing ground locations of bluefin tuna (*Thunnus thynnus*) caught by month as bycatch in southern Gulf of Mexico.

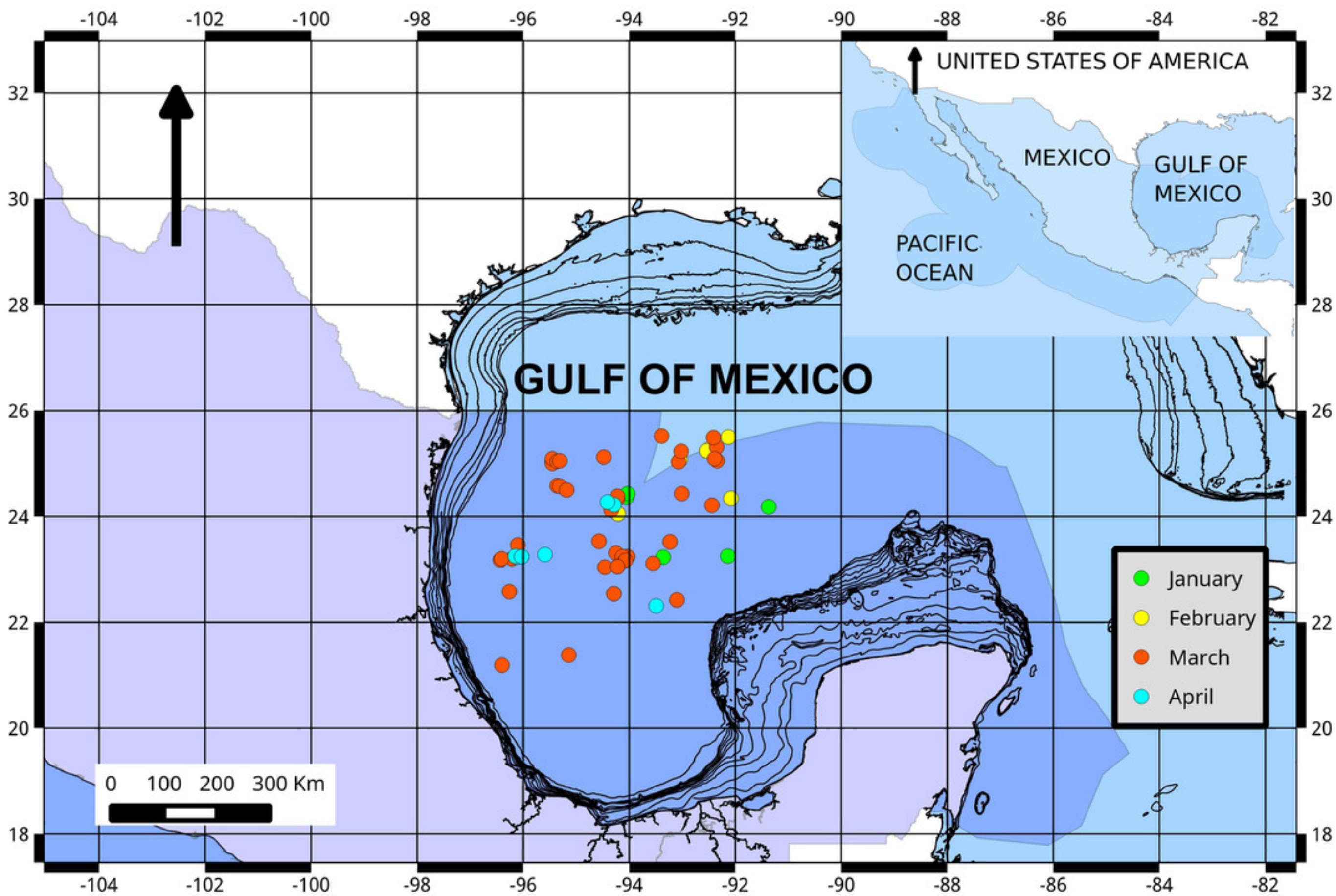


Figure 2

Length distribution of bluefin tuna (*Thunnus thynnu*s) caught by the Mexican longline fleet on 2015.

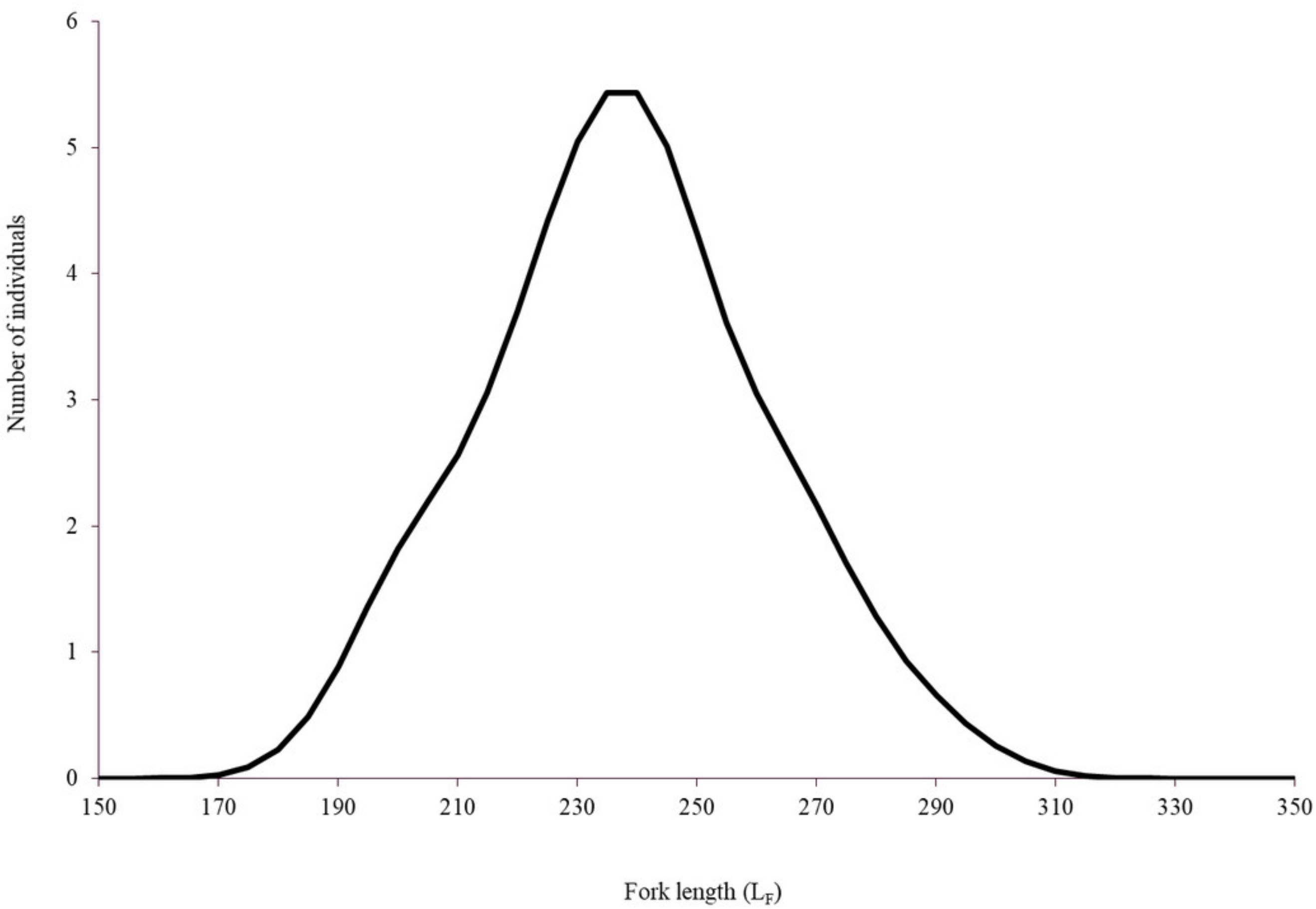
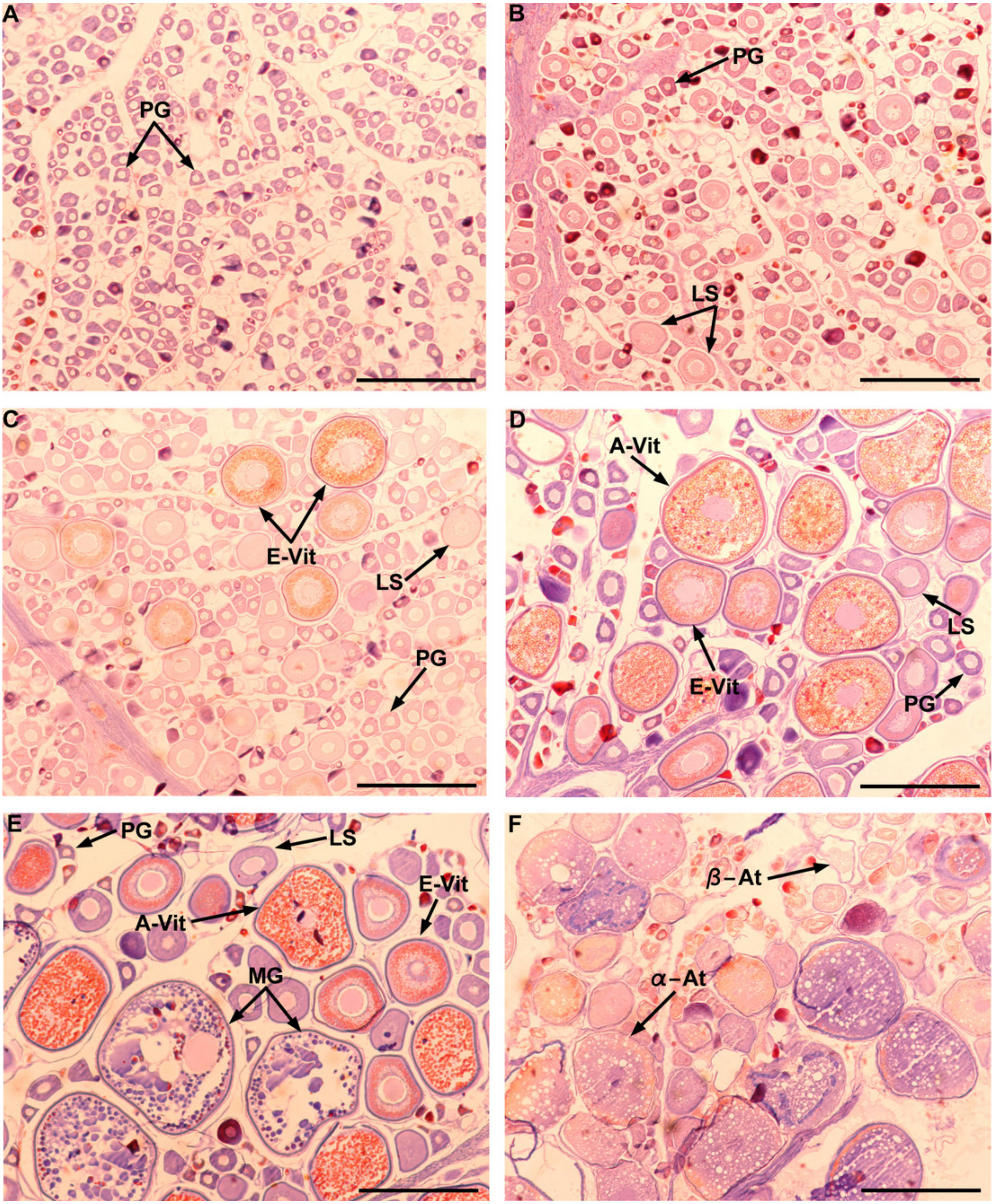


Figure 3

Reproductive stages for ovarian of bluefin tuna (*Thunnus thynnus*) caught in southern Gulf of Mexico.

Ovarian in regenerating (A-B), developing (C), spawning capable (D), spawning (E) and regressing stages (F). PG, primary growth oocyte; LS, lipid-stage oocyte; E-Vit, early vitellogenic oocyte; A-Vit, advanced vitellogenic oocyte; MG, migratory nucleus oocyte; α-At, alpha atresia; β-At, beta atresia. Scale bar = 500 μm.


A
PG
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PG
LS
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D
A-Vit
LS
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PG
E
PG
LS
E-Vit
A-Vit
MG
F
β−At
α−At

Figure 4

Reproductive stages for testes of bluefin tuna (*Thunnus thynnus*) caught in southern Gulf of Mexico.

Testes in early spermatogenesis (A), late spermatogenesis (B), spawning (C) and spent stages (D). GC, germinal cells; SD, spermatids; SZ, spermatozoa. Scale bar = 500 µm.

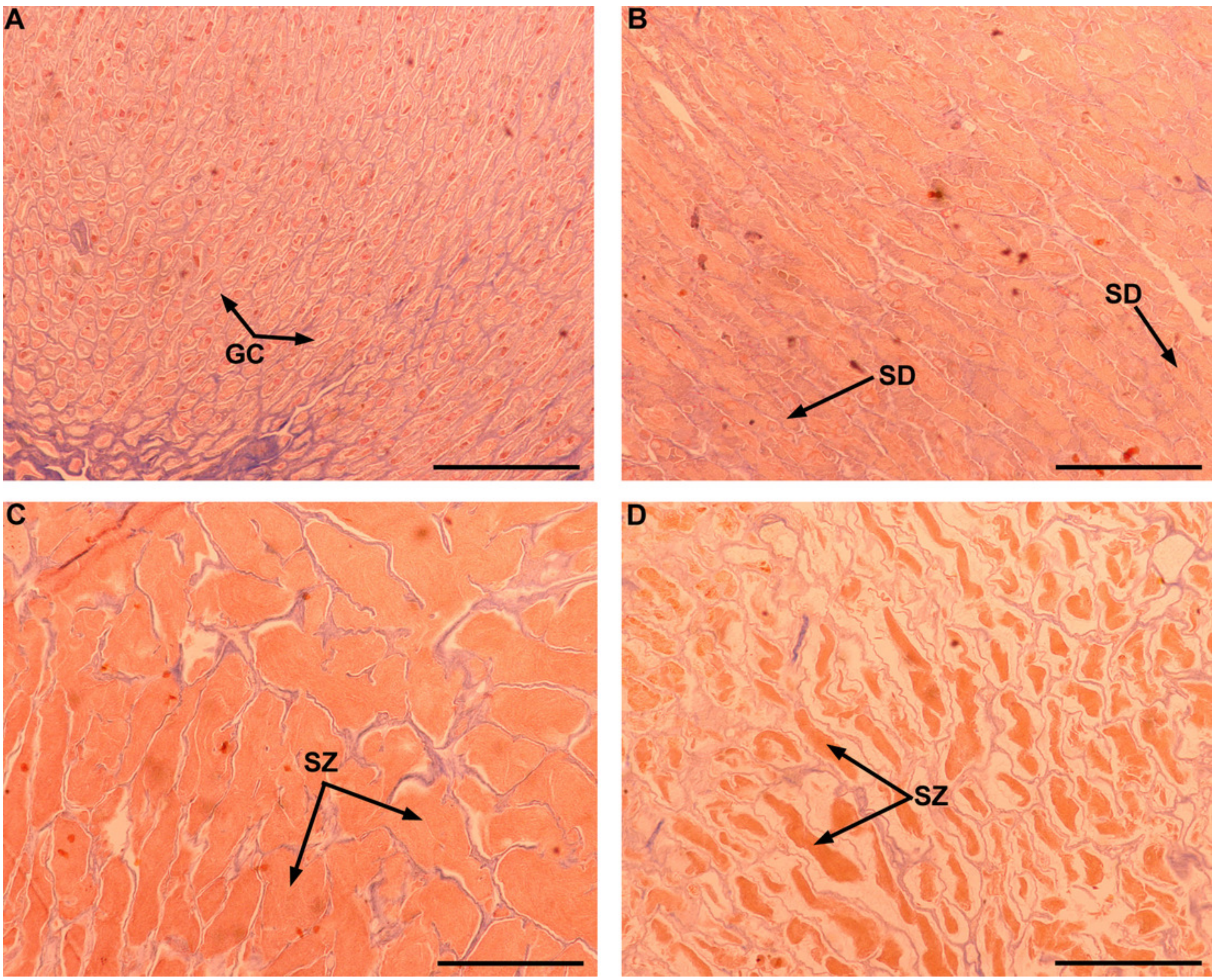

Figure 5

Monthly variation of SST in the area of operation of the Mexican longline fleet targeting yellowfin tuna during 2015.

The red ellipse shows the temperature in the months at which bluefin tuna (*Thunnus thynnus*) were caught.

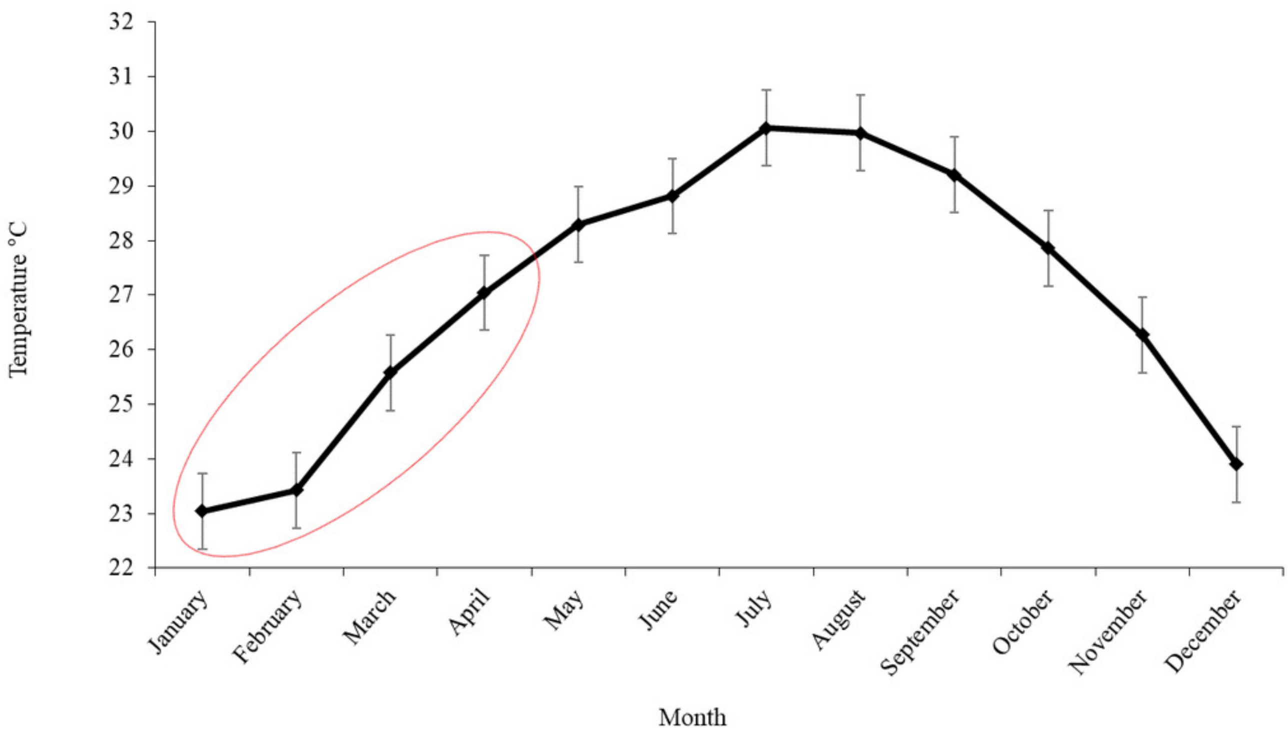

Table 1(on next page)

Temporary progression of reproductive stages in female individuals of bluefin tuna (*Thunnus thynnus*) collected in 2015.

Months were divided in early (from the day 1 to 10), mid (from the day 11 to 20) and late (from the day 21 to 31).

Reproductive stage	Months												Total
	January			February			March			April			
	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late	
Regenerating		1	1			4	3	1	2				**12**
Developing									1	1			**2**
Spawning capable									12	2			**14**
Spawning										1			**1**
Regressing									2				**2**

Table 2(on next page)

Temporary progression of reproductive stages for male individuals of bluefin tuna (*Thunnus thynnus*) collected in 2015.

Months were divided in early (from the day 1 to 10), mid (from the day 11 to 20) and late (from the day 21 to 31).

Reproductive stage	**Months**												**Total**
	January			**February**			**March**			**April**			
	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late	
Early spermatogenesis			1				1						**2**
Late spermatogenesis									5	1			**6**
Spawning								2	1			1	**4**
Spent									1	2			**3**