ALBACORE (*Thunnus alalunga*) REPRODUCTIVE BIOLOGY STUDY FOR THE NORTH ATLANTIC STOCK:YEARS 2020 AND 2021.

Ortiz de Zárate,V[1]., Macías, D[2]., Arocha, F[3]., Su, N-J.[4], Dheeraj, B[5], A. Hanke[5], M. A. Puerto[2], M J. Gomez[2], Parejo, A[1]., Castillo, I[1]

SUMMARY

The ICCAT North Atlantic Albacore (Thunnus alalunga) Research Program was established to enhance knowledge on albacore to provide more accurate scientific advice to the Commission. Funds are provided to the Albacore WG to develop research activities to accomplish several objectives. One of the research objectives is to increase knowledge on reproductive biology for the northern Atlantic stock, maturity schedules (L_{50}) and egg production (size/age related fecundity. In March 2021, Terms of Reference were published by ICCAT. A Consortium integrated by Canada, Venezuela, Chinese-Taipei and Spain presented an offer to collect gonad samples and spines throughout the year and carry out the study of reproductive biology for North Atlantic albacore stock.

Results of histological analysis: maturity stages, batch fecundity and seasonal area of spawners are presented as well as the age determined of partially collection of albacore spines. Analysis were done with the total albacore gonads samples collected in 2020 and 2021 for the reproductive biology study of northern albacore.

RESUMEN

El Programa de investigación del atún blanco (Thunnus alalunga) del Atlántico norte de ICCAT se estableció para mejorar el conocimiento sobre el atún blanco y proporcionar un asesoramiento científico más preciso a la Comisión. Fondos se proporcionan al GT de atún blanco para desarrollar actividades de investigación y alcanzar una serie de objetivos. Entre ellos, se encuentra aumentar el conocimiento sobre la biología reproductiva del stock norte de atún blanco, parámetros como: la talla de primera madurez (L_{50}) y la producción de ovocitos (fecundidad talla/edad). En Marzo 2021, un Consorcio integrado por Canadá, Venezuela, Taipei- Chino y España presentó una oferta para llevar a cabo el estudio de la biología reproductiva del atún blanco del stock norte del Atlántico ofertada por ICCAT.

Se presentan los resultados de los análisis histológicos realizados sobre: madurez, fecundidad parcial y el área y época de los reproductores. Así como la edad determinada en una muestra parcial de individuos muestreados. El total de muestras de gónadas y espinas recogidas durante el otoño de 2020 y el año 2021 fue analizado en este estudio de reproducción del atún balnco del Atlántico norte.

KEYWORDS

North Atlantic, Albacore, Thunnus alalunga, Reproduction, Maturity, Age, Fecundity, Spawning

1 Instituto Español deOceanografía.IEO-CSICApdo.240.39080Santander. Spain.victoria.zarate@ieo.es

2 Instituto Español de Oceanografía.IEO-CSICApdo. 285. 29640 Fuengirola (Malága). Spain. david.macias@ieo.es

3 Instituto Oceanográfico de Venezuela. Universidad de Oriente. Cumaná. Venezuela. farochap@gmail.com

4 NTOU. No2. Beining Rd.Keelung City 202301. Taiwan ROC. nanjay@mail.ntou.edu.tw

5 Department of Fisheries and Oceans.125 Marine Science Drive, St.Andrews, NB Canada. dheeraj.busawon@dfo-mpo.gc.ca

1. Introduction

Albacore (*Thunnus alalunga*) is a highly migratory species (Aloncle et Delaporte 1973; Bard 1981; Ortiz de Zárate and Cort 1998; Arrizabalaga 2003). In the Atlantic ocean two differentiated stocks have been described for assessment purposes (Arrizabalaga *et al*., 2004). Scarce knowledge on the reproductive biology of northern stock is available and still major gaps remained. Within the Working Group of albacore a Research Program of North Atlantic Albacore stock was established and funds were made available by ICCAT.

The albacore (*Thunnus alalunga*) is widely distributed in the Atlantic Ocean where at present two spawning areas have been described based on the distribution of the mature albacore caught by longline (Koto, 1970) and the presence of larvae (Ueyanagi, 1967), corresponding to the north and south stocks being separated by 5 degrees North of latitude. The first studies on reproduction were reported by Beardsley (1969) and Koto (1969), who investigated the location and timing of spawning from samples of adults caught in the Japanese long-line fishery in the late sixties. The spawning area in the northern hemisphere is located in the Sargasso Sea and northern Venezuela and spawning occurs in the boreal summer (Bard, 1974).

Within the Albacore Research Program of ICCAT, it was prioritized to enhance the knowledge of the reproductive biology of North Atlantic albacore stock. A quote was launched by ICCAT in January 2021 and a consortium of researchers developed a description of work to support of the objectives of the Research Program. The quote addresses sampling strategy and analysis for growth, reproduction and maturity.

The objectives of the study are: i) Resolve the age and size at maturity (L_{50}) and size/age related fecundity of Northern Atlantic albacore using samples/measurements provided by participating CPCs, ii) Determine the spawning (areas and season).

Present maturity ogives of albacore are usually estimated using gonadosomatic indices, macroscopic maturity stages, and microscopic examination of gonads (e.g., Arocha and Lee, 1996; Tserpes *et al*., 2001). The most common methods for the estimation of the size at maturity and determine both the spawning period and areas are the gonadosomatic indices and maturity data. However mature gonads in early maturing and recovering stages may appear immature by using those two methods (Schaefer, 2001). The histological analysis of gonads has proved to be the most adequate technique to establish the status of reproductive maturation for tuna species (Schaefer, 1998).

In this study microscopic methods are used to determine the maturity staging of North Atlantic albacore (Saber, S., *et al*., 2016), by analyzing the oocyte development size in the ovaries (Saber, S., *et al*., 2015). Fecundity estimates for North Atlantic Albacore will be done by applying two methods: the Weibel method (Weibel y Gomez, 1962; Weibel, *et al*., 1966; Weibel, E. R. 1969).

Processing and aging of fin ray sections from first dorsal spine collected had followed protocol developed by Ortiz de Zárate *et al*. (2007).

To accomplish the objectives of the Research Reproductive Biological study, a sampling program for collection of biological samples (spines and gonads) for the study areas was designed with the participation of longline fleets from Venezuela and Chinese-Taipei taking albacore as by-catch and as a target species respectively in North Atlantic.

The aim of this document is to present the findings from theNorthern albacore reproductive biology study based on the gonads and spines processed samples that had been collected from the December of 2020 to December 2021.

2. Material and methods

In the study, it was planned where and when to collect the albacore samples in agreement with the commercial fleets that will take scientific observer on board to carry out the task. In **Table 1** is summarized the number of albacore, both female and male, that was ideally expected to be collected along the autumn of 2020 and year 2021. Longline fleets participating in the study are vessels from Venezuela, Chinese-Taipei and Canada. Those fleets catch adult albacore (> 85 cm SFL) that are potential spawners in the tropical areas of central North Atlantic Ocean and Sargasso Sea area. **Figure 1**, shows the spatial stratification sampling areas of North

Atlantic Ocean used in the study. These areas covered the most likely presence of the commercial longliners in the fishing grounds during year round from participants in the Consortium.

All albacore individuals caught were sexed and the straight fork length (SFL) to the nearest centimeter and wet weight (kg) measured. The gonads and the first fin ray of the first dorsal fin were removed from each fish. Each pair of gonads (ovaries, testes) was removed from the gut cavity and weighted wet to the nearest centigram.

From each pair of gonads a 2 cm wide section from the central part of one single gonad (left or right) was removed and preserved in 10 % buffered formalin vial and stored on board the commercial longline vessels until arrival to fishing port. Vials containing samples were delivered to the laboratory in charge of the process and analysis of gonads samples.

Once in the laboratory, gonad subsamples were dehydrated through a series of increasing concentrations of ethanol, cleared with n-butanol, and embedded in paraffin. Histological sections of 10 μm were obtained using a microtome and stained.

2.1. Spines collection and aging method

Spines were collected from albacore at the same time that gonads were sampled. The spines were prepared following the methodology protocol developed by IEO described by Ortiz de Zárate *et al*., 2007. Two consecutive cross cuts of 0.5 mm width were done at the base of each spine condyle, using a low-speed saw (ISOMET). The two sections were then mounted on slides and fixed with a transparent resin covering the surface.

Processing and aging of fin ray sections from first dorsal spine collected had been done by following the protocol developed by IEO (Ortiz de Zárate et al., 2007).

Methodology for reading the spine section was applied such in Ortiz de Zárate and Babcock (2016) and precision and accuracy test was done among two readers involved in the aging of samples (Campana, 2001).

The birth date assumed for North Atlantic albacore stock for aging, is June 1st. Each reader had available the length for each fish. Measurements of the diameter section in the direction of the horizontal axis and the visible annuli were taken by means of a NIKON profile projector with transmitted light using a x10 zoom, and all the annuli identified were counted to determine the age of the fish. However, as the fish grows, the central zone of the spine section is re-absorbed and the first annulus might have been vanished. In this case age of first visible annulus was back-calculated (Ortiz de Zárate and Babcock, 2016).

Each of the two readers read independently the spine sample twice, and then both readers results were compared. The individuals that no agreement was found on aging were jointly read a third time and agreement was reached to determine the final age of a given fish. If still not consensus was reached the experienced reader age results prevailed.

The widely used and statistically sound measures of ageing precision: average percent error (APE) and coefficient of variation (CV) were estimated between the two readers independently age determination.
Several laboratories suggest that a CV of 5% serves as a reference point for many fishes of moderate longevity and reading complexity (Campana, 2001).

2.2 Histological analysis

Assuming a homogeneous distribution of oocytes within and between ovarian lobes, as documented earlier for tunas (Otsu and Uchida, 1959; Stéquert and Ramcharrun, 1995), for each fish sampled a 2 − 3 cm wide cross-section from the central part of one of the lobes was fixed in formalin solution (4% formaldehyde) within 60 ml BiopSafe® vials, and then preserved in 70% ethanol. Then, a representative subsample (from the ovarian wall to the lumen) was taken from each of preserved ovary, dehydrated through increasing concentrations of ethanol series, cleared with n-butanol, and embedded in paraffin.

Sections were cut at 10 µm and stained with Mallory's trichrome stain. All images were taken on a Nikon photomicroscope with either x10, ×4 or ×2 objective magnifications. Significant differences in the distribution of oocytes among the centre, mid-region and periphery of the same albacore ovarian section has been found (Otsu and Uchida, 1959). Thus, in order to avoid biased estimations due to these transversal structural differences affecting oocyte distribution, the set of micrographs of each ovary was taking randomly but representing the whole ovarian section. The resulting area of each micrograph was 3.323 mm2 for ×4 objective magnification that we used to ulterior batch fecundity BF estimates.

According to their different developmental stages, oocytes were classified into one of 6 classes using similar terminology of Brown-Peterson et al. (2011), namely: primary growth (PG), cortical alveolar (CA), Early vitellogenic (EVO), advanced vitellogenic (AVO), migratory nucleus (MG) and hydrated oocyte stages (HY) as detailed in the **Table 2**.

All histological sections and microphotographs were read by two readers. Each ovary was examined to record the most advanced group of oocytes (MAGO), the presence of: postovulatory follicles (POFs), atretic follicles (alpha or beta atresia) and, maturity markers. The maturity markers were well defined muscle bundles and numerous brown bodies (Farley et al., 2016). Subsequently, histological criteria based on a modification of the criteria of Schaefer (1998) and Farley et al. (2013) were applied to determine the maturity status of each female and its ovarian phase. This new scale divide the stage II (developing) into two new substages: Substage IIa (developing coming from stage I) and Stage IIb (developing coming from Stage Vb). For more detailed description see **Table 3.**

A logistic regression model was fit to the proportion of mature fish (the probability of maturity (p) versus length), assuming a binomial error distribution. Confidence intervals for the parameters of the logistic regression were estimated using bootstrapping.

To calculate the Gonadosomatic Index (GSI), the following equation (Gibson and Ezzy, 1980) was used. The equation helps monitor monthly changes in gonad development that indicate reproductive activity
GSI = GW /(RW-GW) × 100
where GW is gonad weight and RW is round weight of the sampled individual.

Spawning season and annual maturity cycle have been analyzed using the proportion of gonads stages by month.

Batch Fecundity estimates.

The numerical density (Nv) of the particles of interest, i.e. the number of MG oocytes per unit volume, was calculated according to principles first developed by Weibel and Gomez (1962) but using here the further modified formula in Weibel et al. (1966). The W&G stereological method includes "the Delesse principle", which states that the fractional volume (Vi) of a component i in a given tissue (here fish ovary) is proportional Ai) (Emerson et al., 1990). The resulting main W&G formula reads:

$$N_V = \frac{\Sigma Q^-}{(\Sigma P \times a/f \times h)}$$

where k is the MG oocyte size distribution coefficient, β the MG oocyte shape coefficient, Na the average number of MG oocytes per unit of area, and Vi the average volume fraction occupied by MG oocytes.
Batch fecundity (BF) was defined as the total number of MG (migratory nucleous) oocytes per spawning batch. Then, the given number of oocytes per unit volume, Nv, was multiplied by the whole ovarian volume. Using Scherle's method (1970) the ovaries showed a mean loss in tissue volume of 12.4% when dehydrated in 99.6% ethanol. Hence, ovarian volume was corrected for shrinkage, and these corrected ovarian volume values were thereafter incorporated in the estimations of batch fecundity (Knapp et al., 2014).

The relative batch fecundity (BFrel) was calculated by dividing the batch fecundity by the body weight of the fish.

3. Results

The total number of samples collected are resumed in **Table 4**. It is observed that the contribution to the

collection of gonads of the different fleets in comparison with the planned design (see **Table 1**) for the reproductive study diverge. The availability of the resource in the spatial/temporal strata where commercial fleets operate in North Atlantic was difficult to forecast and match for some fleets, mainly Canada and Chinese-Taipei longliners. At the contrary collection of samples by Venezuela longline fleet exceeded the planned number of fish to be sampled. The realized samples by Venezuela were n= 177, adding n= 79 individuals collected by Chinese-Taipei and none by Canada fleet. From Chinese-Taipei total sample collection of 6 individuals were left out due to incomplete information, and were discarded for the analysis. The effective number for the study was n= 73 individuals(**Table 4).**

Chinese-Taipei longline fleet albacore samples were collected in May and June in and came from the in areas 2 and 4, around the parallel 20 º N in Central North Atlantic, where this fleet mainly operates. The Venezuelan fleet caught albacore in areas 3 and 4, located around the parallel 10º and in area 5 in the Caribbean Sea, relatively close to coastal areas as shown in **Figure 2,** where it is illustrates the proportion of female and male albacore collected by each longline fleet centered at 1ºby 1º degree position.

Size (SFL cm) frequency distribution of albacore both female and male fish aggregated that were analysed are presented in **Figure 3**. It is observed that all individuals are adult albacore (> 90 cm SFL). Larger sizes were sampled by Chinese -Taipei in central North Atlantic in May and June 2021.

Monthly mean size distribution for both female and albacore fish collected by Venezuela longline fleet during 2020 and 2021 in areas 3, 4, and 5 .are shown in **Figure 4**. It is observed that all individuals are adult albacore (> 90 cm SFL). M male individuals are of larger sizes than females overall the study time but March and May.

Complete monthly mean size distribution for both female and albacore fish collected by Venezuela and Chinese longline fleets during 2020 and 2021 in areas 2, 3, 4, and 5 .are shown in **Figure 5**. The larger female are encounter in May and June sampled by Chinese-Taipei fleet.

3.1 Spines collection and aging method

The results of the final ages assigned by the two readers in agreement resulted in aging the complete set of spines (n=163) The average percent error (APE) between the two readers and the precision expressed by the coefficient of variation (CV) were estimated to be 4.842 and 6.847, respectively (Ortiz de Zárate *et al*., 2021).

The total number of albacore spine collection (n=163) obtained by implementing the Venezuela sampling plan in 2021was analized for age determination.The results of the final ages assigned the two readers agreement in the complete set of spines (n=163) by month and sex is presented in **Table 5**. All months were covered but January and October, when samples were not accomplished.

In **Table 6** are summarized the results of mean size (SFL) and SD by sex and age group determined. Females ranged from 4 to 10 years and males were determined for age groups ranging from 5 to 8, with scarce representation of age groups 5 and 8. Likewise, very few samples were available for larger size females albacore individuals, ages 9 and 10 and in the case of the youngest female individuals age 4.. Number of samples across month is reduced specially in May, June and August.

Complementary information on length distribution by age group determined and sex information is presented in **Figure 6**. It is observed larger sizes for male individuals in age groups: 6, 7 and 8.

3.2 Histological analysis

The total amount of gonads (*n*=272) were processed at the laboratory in charge. The samples obtained in 2018 were at immature histological stage-

The 2020 and 2021 albacore fish collected (n=264) were analysed histologically, and the stage determined microscopically. The results are shown in **Table 7** for: 213 female and 51 male. Sixty-eight-six fish (55 female and 13 male) came from the study area 5 and 196 (158 female and 38 male) from the study areas 2, 3 and 4 (see **Figure 2**).

The majority of the fish analyzed (n=110) were in recovering stage (gonad stage Vb). We have found 84 active fish (stage III and IV) in the sample (Table 7). Only 12 active fish (Stage III) came from the Venezuela sampling

area. No fish in spawning stage (Stage IV) were observed in Venezuela sampling areas (**Table 8**). The majority of active fish (7 stage III and 65 of stage IV) come from Chinese-Taipei sampling developed in the northwestern of the area 4 and the southwestern of area 2 (**Figure 2**). The **Figure 7** shows microphotographs of some of the ovarian analyzed from the Venezuela sampling area. Note an immature ovary in the **Figure 7A**, a developing ovary in the **Figure 7B**, and the active fish (Stage III) in the **Figure7C** and **D**. The **Figures 7E** and **4F** show postspawning ovaries: Stage Va (regressing) and Vb (recovering), respectively.

In the **Figure 8** is presented an active ovary coming from Chinese-Taipei sampling area. It shows an active fish (Stage IV) with that was found with migratory nucleus oocytes stage (MG oocytes).

Size at First Maturity (L50)

After the microscopic analysis of maturity status of the 264 fish, it was observed that only 8 fish were immature (7 female and 1 male), so it was impossible to adjust the logistic curve to obtain the maturity ogive. In this context, any estimates of L50 can be done unless new immature specimens of smaller length (< 90 cm FSL length) can be sampled in the Atlantic Ocean.

Fecundity

Twenty-one fish (*n*=21)were selected for batch fecundity (BF) estimation, with size ranging from 102 to 111 cm SFL. The results of the estimates using the W&W method and counting MG oocytes **.**

The mean batch fecundity (BF) estimate was 1.28 million oocytes, ranging from 0.58 to 2.23 millions oocytes. A preliminary correlation test suggested that the fecundity could be positively correlated with the size, the body weight and the gonad weight, but the size of the study sample is considered low to obtain statistical significance.

The average relative batch fecundity (BFrel) estimation was 54.3 oocytes per gram of body weight, ranging from 30.4 to 92.8. These values are in the range of estimates for other albacore stocks.

Spawning season

Figure 9 shows the different monthly maturity stages found in the data set analysed Active fish (Stages II and IV) are found mainly in May and June, but also can be found some fish in spawning capable stage (III) in February, March, July and September. The majority of the fish analyzed were inactive fish mainly in recovery stage (Vb; n=110)**.**

Spawning fish have been identified in the samples available from Chinese-Taipei taken in northwestern of area 4 and the southwestern of area 2 **(Figure 2**) in May and June (**Figure 10**).

Spawning fish have not been identify in the samples available from Venezuela taken in area 5 the Caribbean and in areas 3 and 4 located in offshore East Northern coast of Venezuela (**Figure 2**). Only a few active fish (Stage III) were found in February, March, May, July and September. The majority of the fish analyzed were inactive fish mainly in recovery (stage Vb) and developing stage (IIb).

The analysis by sex indicates that spawning capable female (stage III) are found in February, May and June. Male fish in stage III (advanced spermatogenesis stage) are found in March, May, June, July and September. Male fish in spawning stage (IV) are found in May and June. In the areas 2 and 4 can be found both females in spawning capable (February, May and June) and spawning (May and June) stages (**Figure 10**).

The percentage of North Atlantic albacore female by gonad stage sampled in areas 2, 3 and area 4 in Central North Atlantic by month and sampling areas are shown in **Figure 11**.

Regarding the gonad stage of female by areas, in the area 5 the spawning capable female (Stage III) only appears in May. Any spawning female have been detected in this area. Closer examination of the histological stage results indicated that the majority of the females in area 5 were in the recovery stage (**Figure 2**).

On the other hand, in area 3 only can be found female fish in stage III in February. It is important to note that the temporal distribution of the sampling size of Stage IV is narrow and that spawning females could be present in the northwestern of area 4 and area 2 for a more extended period. There is a significant temporal gap in sampling for these spawning area with no samples collected from July to May (**Figure 10**).

The analysis of GSI by gonad stage agreed with previous works in the tuna species with an increasing trends on mean GSI values from developing (IIa, IIb) to the spawning capable stages (peak GSI values) and a decreasing trend from spawning capable (III) to recovering stages (Vb).

4. Discussion

Arrange the sampling of albacore gonads in the North Atlantic has been challenging . This study results have proven to be feasible and with the accumulated experience acquired by the Consortium new sampling can be implemented to continue to complete knowledge on the reproductive biology of albacore.

The sample size has prevented to obtained more descriptive results on the reproductive biology of North Atlantic albacore and to do statistical analysis.

Nevertheless it represents an step forward on new findings about the area, season and fecundity of the Northen albacore stock not described before.

The monthly variation of the mean GSI values (GSI) suggest that the spawning season extends at least from May to June, but probably extends for a more extended period (March to August) attending to the presence of spawning capable females from February to September in the area 4 (**Figure 2**). Male fish in spawning stage (IV) are found in May and June in Central North Atlantic area around the parallel 20º N (**Figure 10**).

In the areas 2 and 4 can be found females in spawning capable stages in February, May and June (**Figure 10**) and spawning fish have been identified in the samples available from Chinese-Taipei taken in northwestern of area 4 and the southwestern of area 2 (**Figure 2**) in May and June.

The mean batch fecundity (BF) estimate was 1.28 million oocytes, ranging from 0.58 to 2.23 millions oocytes for the study are in Central North Atlantic.
The South Pacific albacore batch fecundity (BF) estimated by Farley *et al.,* (2013) ranged from 0.26 to 2.83 million oocytes with a mean relative batch fecundity of 64.4 oocytes per gram of body weight.

The North Pacific Albacore analyzed by Chen *et al.*, (2010) presented batch fecundity (BF) estimates that ranged between 0.17 and 1.66 million eggs. The mean relative BF estimation was 50.5 oocytes g−1 body mass (± 22·8).

Saber *et al*. (2016) studying the BF of the Mediterranean albacore obtained a mean value of 1.01 million oocytes using the W&G Method and counting MG oocytes.

In any case we should increase the sample size in order to test for factors affecting batch fecundity such as the size, body weight, gonad weight, season and area.

A preliminary correlation test suggested that the fecundity could be positively correlated with the size, the body weight and the gonad weight, but the number of samples is considered low to obtain statistical significance.
The average relative batch fecundity (BFrel) estimation was 54.3 oocytes per gram of body weight, ranging from 30.4 to 92.8. These values are in the range of estimates for other albacore stocks.

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Table1. Number of fish planned to be sampled during the reproductive biology study of North Atlantic albacore (*Thunnus alalunga*) stock, years 2020 and 2021.

		CPC	Venzuela BY-CATCH		Canada BY-CATCH		China-Taipei TARGET		Total fish
Year	**Fleet**	**Fleet**	**Longline**		**Longline**		**Longline**		
	Quarter	Month	Female	Male	Female	Male	Female	Male	
2020	4 th Q	December	9	5					14
2021	1st Q	January	13						13
2021		February	22	5					27
2021		March	22	5					27
2021	2nd Q	April	22	5	5	2	30	3	67
2021		May	22	5	5	2	30	3	67
2021		June	22	5	5	2	30	3	67
2021	3rd Q	July			5	2			7
2021		August			5	2			7
2021		September			5	2			7
2021	4th Q	October			5	2			7
2021		November			5	2			7
2021		December							
Total			**132**	**30**	**40**	**16**	**90**	**9**	**317**

Table 2. Description of the oocyte stages in albacore ovaries according to their histological characteristics.

Stage oocytes	Histological characteristics
Primary growth (PG) oocytes	Homogeneous basophilic cytoplasm with no cytoplasmic inclusions.
Cortical alveoli (CA) oocyte	Small lipid droplets in the cytoplasm but still no yolk granules.
Early vitellogenic oocyte (EVO)	Yolk granules occupy only partially the citoplasm. Yolk granules distributed in the periphery of the cytoplasm and lipid droplets occupy the inner cytoplasmic area.
Advanced vitellogenic oocyte (AVO)	Yolk granules and lipid droplets are spread throughout the cytoplasm. Larger yolk granules than that of Early vitelogenic oocytes. In a later phase, Lipid droplets fuse and are distributed around the nucleus.
Migratory nucleus (MG) oocyte	Lipid droplets fuse into 1−3 large droplets. Migration of the nucleus toward the animal pole. In a later phase, yolk granules fuse progressively.
Hydrated (HY) oocyte	The nucleus has disintegrated. All yolk granules fuse into a homogeneous yolk mass and the oocyte increases in size due to hydration. The oocyte is still surrounded by the follicle layer, i.e. ovulation has yet not taken place.

Table 3. Microscopic maturity scale for albacore female based on a modification of the criteria of Farley *et al*. (2013). POFs, postovulatory follicles; MAGO, the most advanced group of oocytes; VO, Vitellogenic Oocytes.

STAGE	MATURITY STATUS	PHASE	MAGO AND POFs	ATRESIA OF VO	MATURITY MARKERS
I	Immature	Immature	Previtelogenic O. No POFs	no atresia	Absent
IIa	Immature	developing	Early vitelogenic O. No POFs	no atresia	Absent
IIb	Mature	developing	Early vitelogenic O. No POFs	late stages of atresia	Present
III	Mature	Spawning capable	Advanced vitelogenic O. no POFs	α and β atresia may be present	Posible
IV	Mature	Spawning	AVO + POFs Migratory nucleous ± POFs Hydrated O. ± POFS	α and β atresia may be present	Posible
Va	Mature	Regresing	Previtelogenic O. or vitelogenic O.	The majority (>50%) of the vitelogenic O. are in a and b atresia	Present
Vb	Mature	Regenerating	Previtelogenic O.	late stages of atresia may be present	Present

Table 4. Monthly gonads sampled obtained by Venezuela and Chinese-Taipei fleets during December 2020 and January-December 2021 for the study.

	Fleet Venezuela						Fleet Chinese-Taipei			Total Study		
	2018	2020		2021		Total	2021		Total	2020 & 2021		Total
	Sex	Sex		Sex			Sex			Sex		
Month	F	F	M	F	M	F & M	F	M	F & M	F	M	F & M
1				13		13				13		13
2				22	5	27				22	5	27
3				8	3	11				8	3	11
4				9	5	14				9	5	14
5				2	2	4	30	7	37	32	9	41
6				3		3	29	7	36	32	7	39
7				36	5	41				36	5	41
8	8			6	5	11				6	5	11
9				21	13	34				21	13	34
10						0						0
11				11		11				11		11
12		9	5	8		8				8		8
Total fish (*n*)	8	9	5	139	38	177	59	14	73	198	52	250

Table 5. Monthly distribution of albacore individuals summarized by age group

Month	2		3		4		5		6		7		8		9		11		12		Total
Age Group	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	n
4	2														1						3
5	9		3	1	3						3	1	1	2	6	1	2		2		34
6	8	1	5	2	1	3	1	1	1		14	1	2	1	10	5	3		3		62
7	3	4			4	2	1	1	1		12	3	2		4	5	5		3		50
8											6			2		2					10
9					1				1		1										3
10													1								1
Total	22	5	8	3	9	5	2	2	3		36	5	6	5	21	13	10		8		163

Tabla 6. Venezuelan aged albacore (*Thunnus alaunga*) samples. Mean fork length (SFL) at age and sex, number of fish aged (n) and standard deviation (SD) .

	Venezuela 2021					
	Males			Females		
Age	Mean SFL (cm)	N	SD	Mean SFL (cm)	N	SD
4				95,67	3	2,08
5	96,00	5	5,24	98,07	29	3,39
6	97,00	14	2,97	99,04	48	3,24
7	104,53	15	4,98	100,31	35	2,27
8	108,00	4	3,16	99,33	6	2,66
9				102,00	3	2,65
10				115	1	0
Total	99,30	38	3,62	102,18	125	5,37

Table 7. Results on Gonad staging of Atlantic albacore by month.

Month/Gonad Stage	I	IIa	IIb	III	Va	Vb	Total
1			2		1	10	**13**
2			4	2	4	16	**27**
3	1		4	3	1	2	**11**
4			4			5	**14**
5			1	2		1	**4**
6					1	2	**3**
7	3		9	4	2	23	**41**
8	2				4	11	**19**
9		1	3	1	15	14	**34**
11		1	2			8	**11**
12			2		2	18	**22**
Total	**6**	**2**	**31**	**12**	**30**	**110**	**199**

Table 8. Results on Gonad staging of Atlantic albacore by month sampled by Venezuela.

Month/Gonad Stage	I	IIa	IIb	III	Va	Vb	Total
1			2		1	10	**13**
2			4	2	4	16	**27**
3	1		4	3	1	2	**11**
4			4			5	**14**
5			1	2		1	**4**
6					1	2	**3**
7	3		9	4	2	23	**41**
8	2				4	11	**19**
9		1	3	1	15	14	**34**
11		1	2			8	**11**
12			2		2	18	**22**
Total	**6**	**2**	**31**	**12**	**30**	**110**	**199**

Figure 1. Spatial distribution of sampling areas defined in the study in 2020 and 2021 for longline commercial fisheries taken albacore either by-catch or targeting.

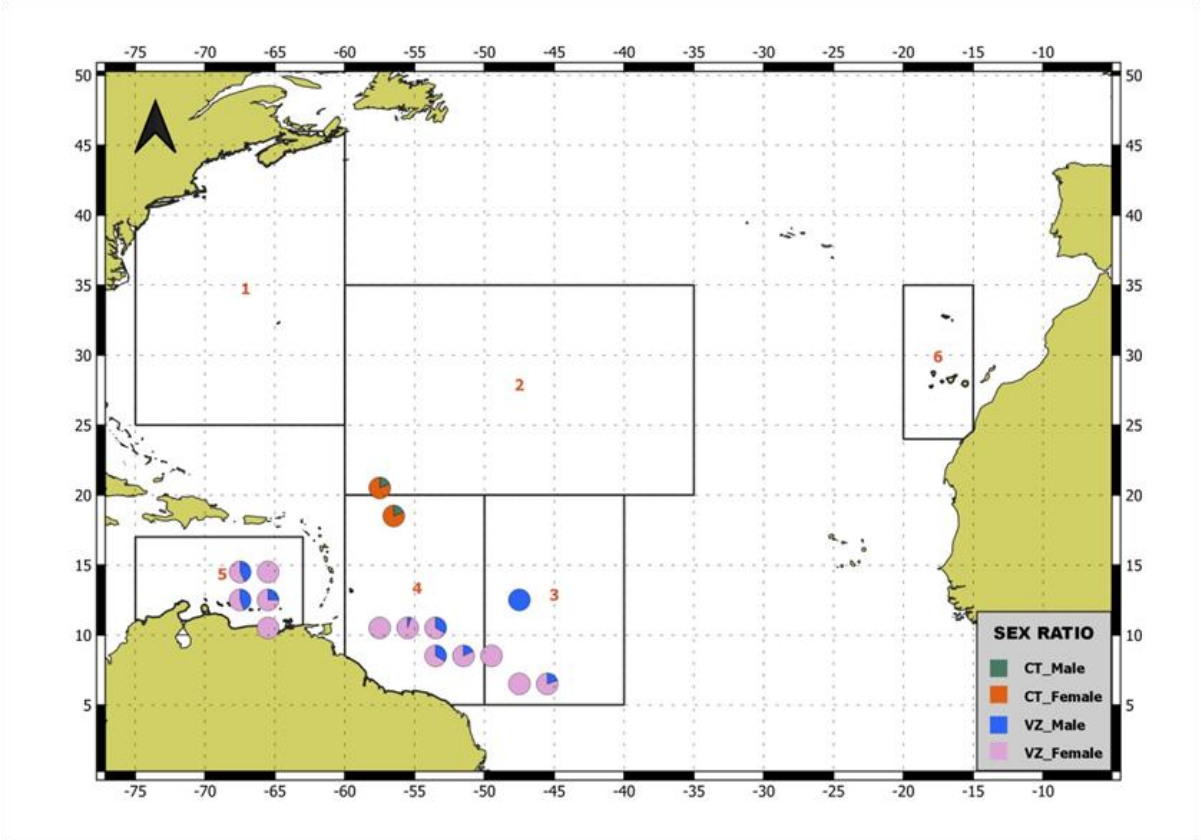

Figure 2. Distribution of proportion of albacore samples by sex obtained for the two longline fleets: Chinese-Taipei and Venezuela.

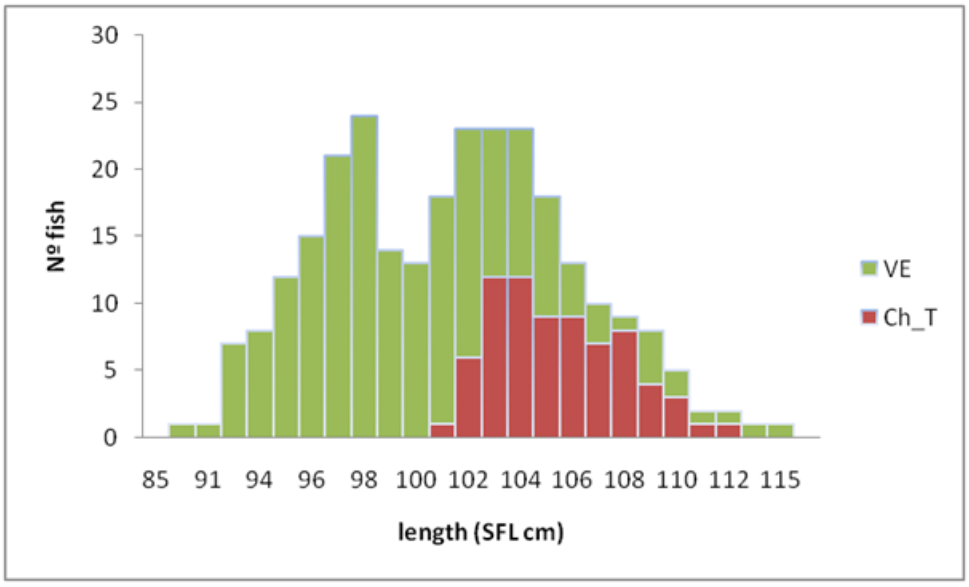

Figure 3. Total length frequency (SFL, cm) distribution of albacore collected by Chinese-Taipéi and Venezuela longliner fleets

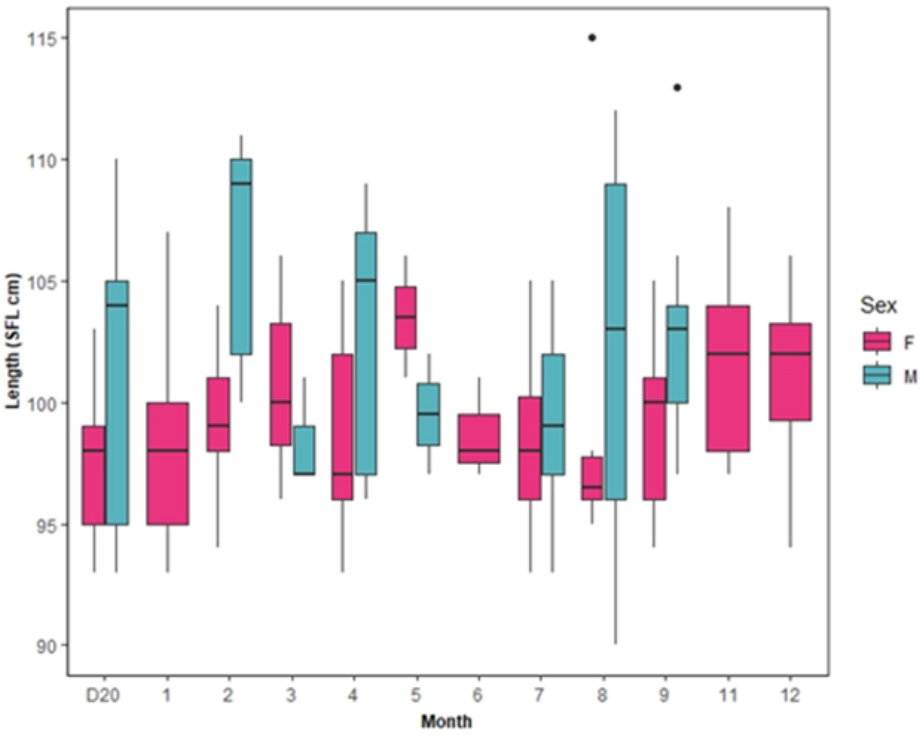

Figure 4. Monthly mean size by sex distribution of albacore samples collected in the study area (4 & 5) by Venezuela longliners during December of 2020 and 2021.

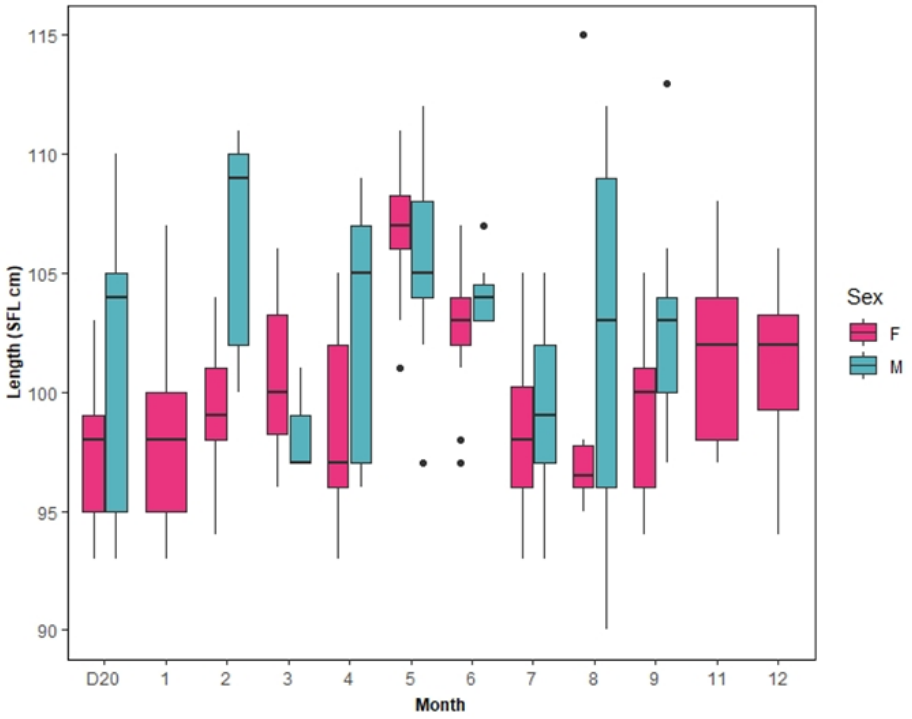

Figure 5. Aggregated monthly mean length (SFL) albacore distribution by sex (F, females and M, males) collected in the study area (4 and 5) by Venezuela longliners during December of 2020 and 2021. Included the samples collected by Chinese a-Taipei in May and June 2021in area 2 and 4.

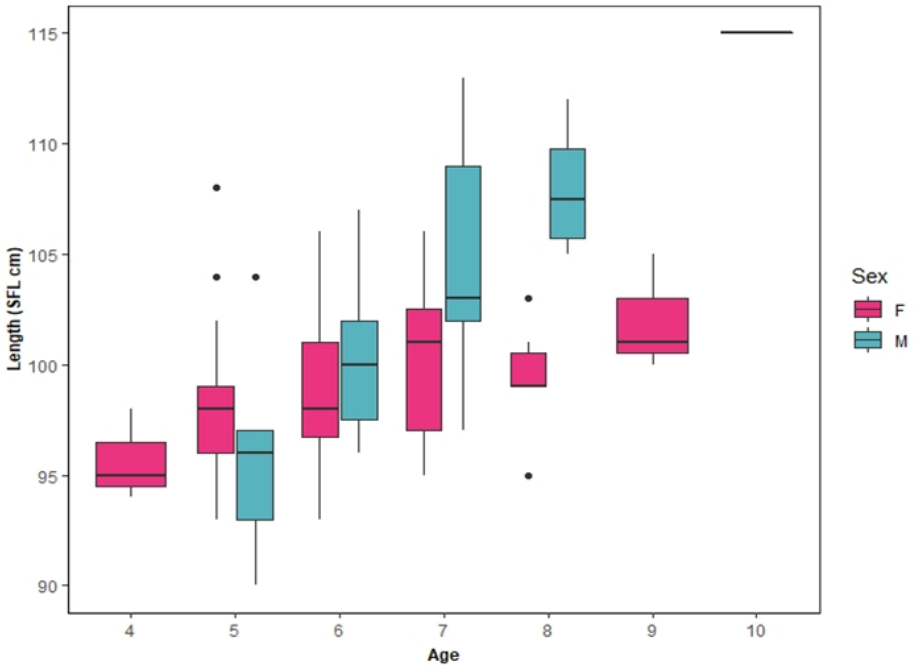

Figure 6. Mean Length distribution of albacore aged by sex (F, females and M, males) collected by Venezuela in 2021.

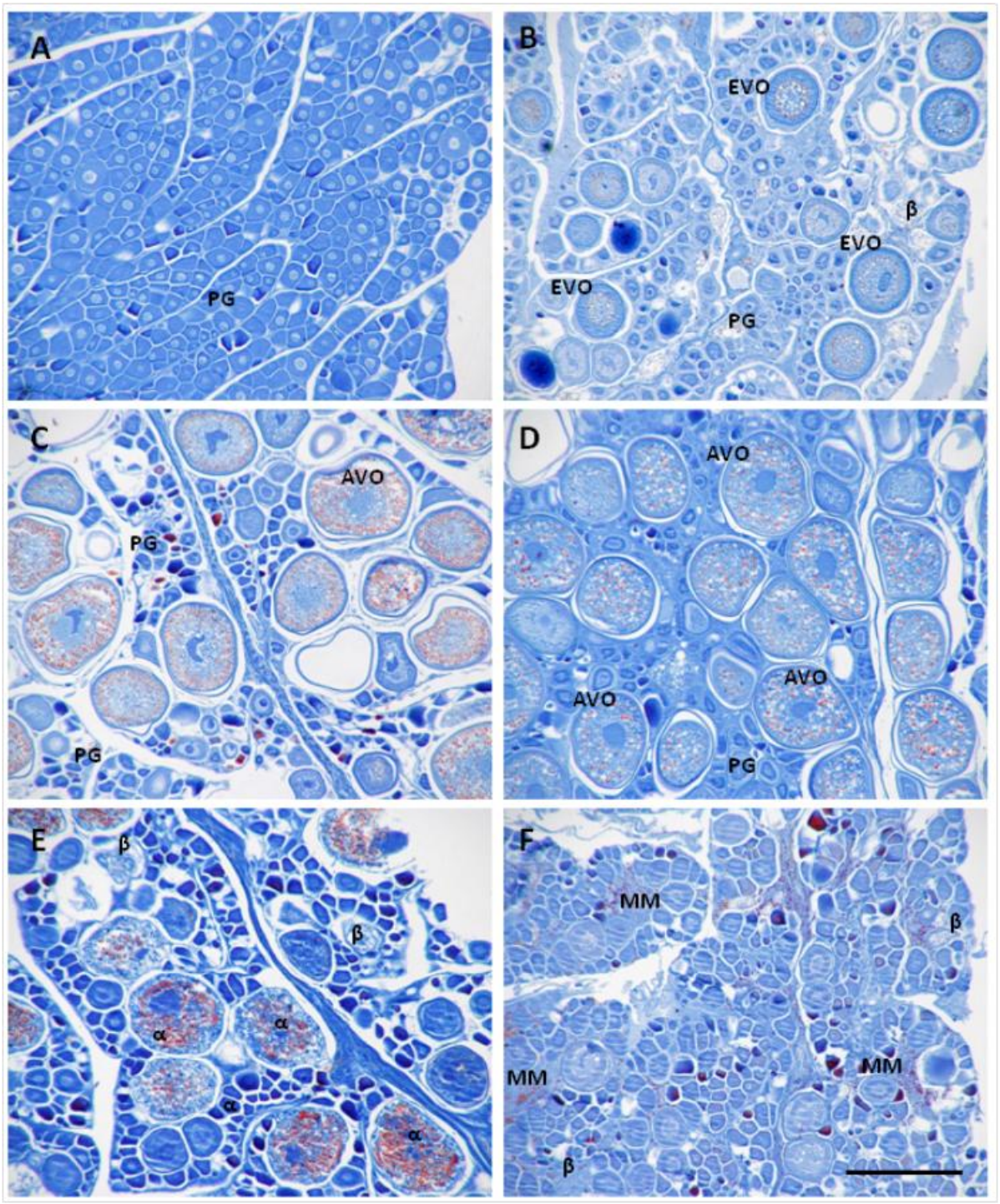

Figure 7. Microphotografhs of gonad stages of Atlantic albacore observed in this study (bar = 500µm). **A.** Image of an immature ovary. PG = Primary Growth Oocytes **B.** Image of an developing ovary. EVO = Early Vitellogenic Oocytes . C and D. Images of Spawning Capable ovary. AVO = Advanced Vitellogenic Oocyte. E. Image of a regressing stage ovary (stage Va) with more than 50% of Vitellogenic oocytes in α atresia (α). F. Image of an regenerating ovary (stage Vb) with some rest of β atresia (β).

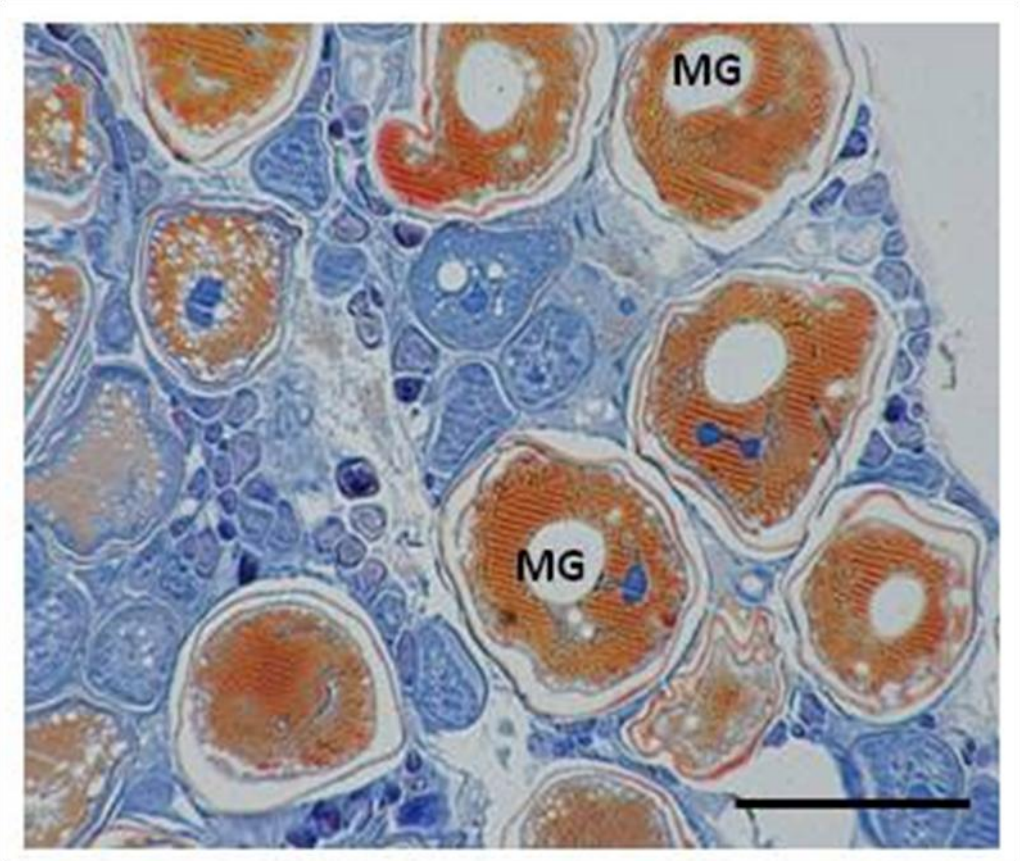

Figure 8. Microphotographs of gonad stage IV (Sapwning) of Atlantic labcore observed in this study study (bar = 500µm). The Image shows a spawning ovary with MG oocytes. MG = Migarory Nucleous Oocytes

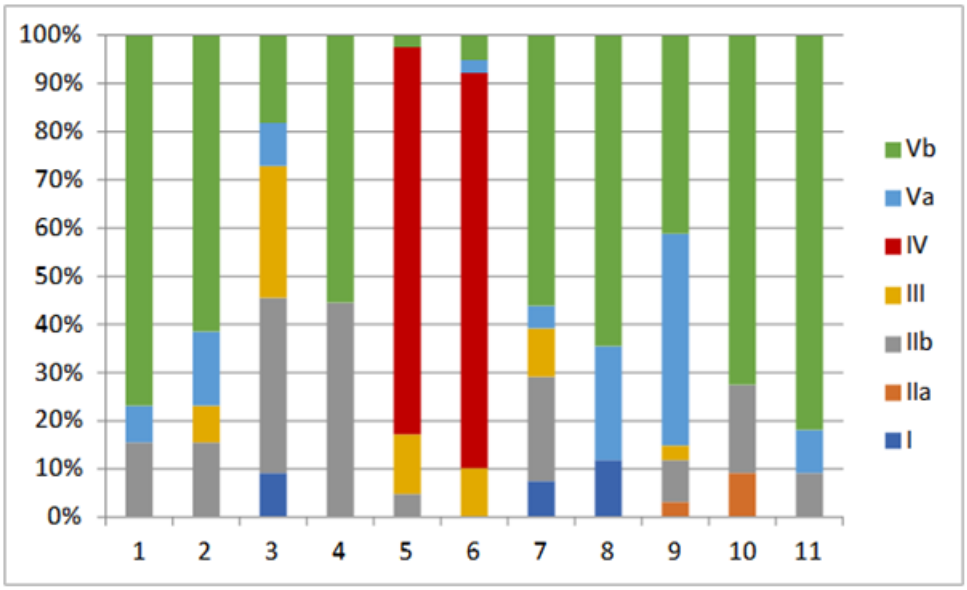

Figure 9. Percentage of North Atlantic albacore by gonad stage found in this study.

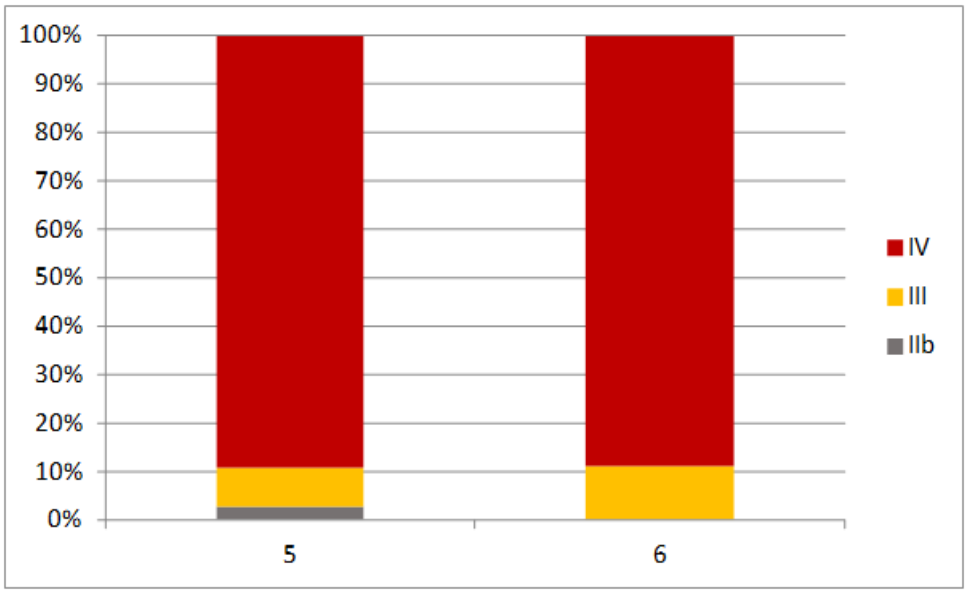

Figure 10 Percentage of North Atlantic albacore by gonad stage from Chinese-Taipei sampling.

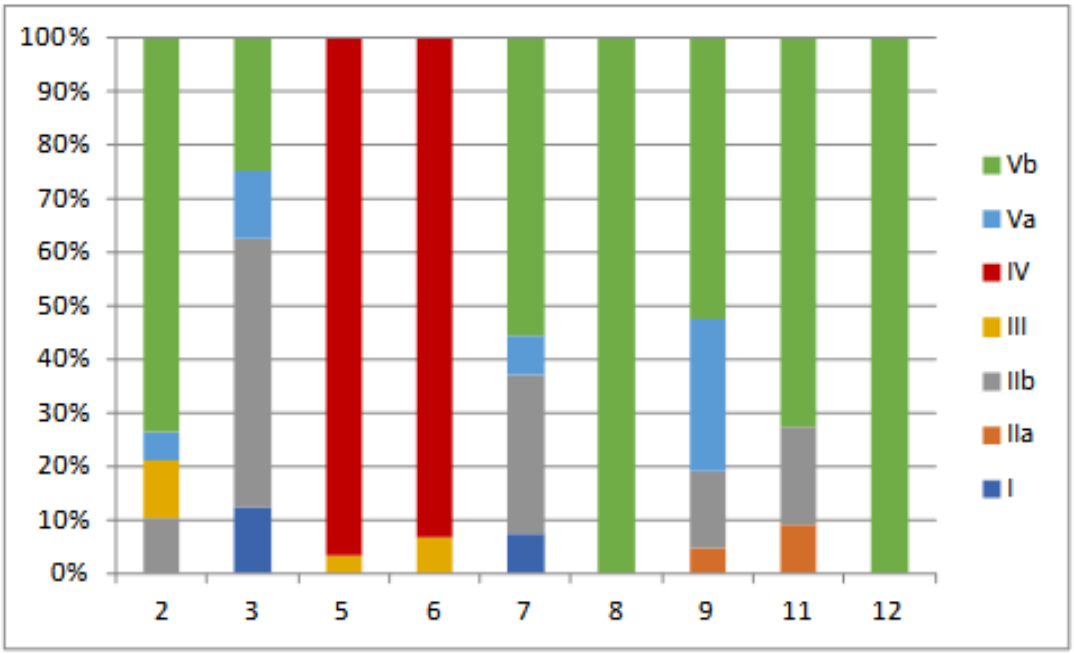

Figure 11. Percentage of North Atlantic albacore female by gonad stage sampled in areas 2, 3 and area 4 in Central North Atlantic sampling areas .