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PRELIMINARY STUDY OF AGE, GROWTH, AND SPAWNING ACTIVITY OF ALBACORE IN AUSTRALIA'S EASTERN TUNA & BILLFISH FISHERY

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Preliminary study of age, growth, and spawning activity of albacore in Australia's Eastern Tuna & Billfish Fishery

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Executive Summary

This report presents the results of a 1-year preliminary study to investigate the size, age and reproduction of albacore caught in the Eastern tuna and Billfish Fishery (ETBF) on Australia's east coast. The objectives of the study were to provided early information on key biological parameters including length-weight conversions, sex ratios, spawning frequency, batch fecundity, and a maturity schedule.

Biological sampling of albacore was undertaken at three locations: Mooloolaba, Cairns, and the east coast of Tasmania. A total of 232 fish were measured for length (212 with sex identified), and 174 gonads, 149 otoliths and 189 fin spines were obtained. Although the sample size was small, the study found that longline catches off Queensland were dominated by adults (>80 cm FL) and the majority were male (70%), while recreational catches off Tasmania were dominated by juveniles (<75 cm) with a sex ratio of 1:1.

Age was estimated independently using otoliths and fin spines for 100 albacore. Both structures had clear growth increments that provide reproducible counts. A direct comparison indicated very little bias in assumed ages 1-7 years, but otolith-based age estimates were generally higher than the spine-based estimates after age 7. Despite this, preliminary von Bertalanffy growth curves obtained from both structures were very similar, and both indicated growth and $L\infty$ was greater in males than females. A maximum age of 14+ and 11+ years was obtained from otoliths and fin spines respectively.

Analysis of 145 gonads collected from ~15-20ºS provided the first record of actively spawning albacore from the Coral Sea, and the high incidence of actively spawning fish during January/February suggests that this is an important spawning area/time. However, the presence of a number of post-spawning females during these months suggests that individuals may not spawn for an extended period. The relatively large number of regenerating females caught in June and July off Queensland also suggests that some females remain in the spawning area after spawning, while others move south as indicated by the presence of a regenerating female off Tasmania in April. Our study confirmed that albacore is a multiple batch spawner with indeterminate annual fecundity. Histological analysis of ovaries confirmed that females are capable of spawning daily, although the average mature female spawned every 1.3 days (in Jan/Feb). Batch fecundity estimated for 7 females ranged from 0.86 to 1.33 million oocytes. We suggest that gonads collected from the temporal window of January to July will allow for a clear distinction between mature and immature females for size at 50% maturity estimation using the presence of atresia (alpha to delta) in histological sections to distinguish immature from post-spawning females.

Substantially more (validated) direct age and reproductive data are required to fully investigate age, growth and reproduction of South Pacific albacore. A regional project is currently in development to expand on this preliminary work.

1. Introduction

The catch of albacore by longliners in the Eastern Tuna and Billfish Fishery (ETBF) reached a record level of 2,583 tonnes in 2006. This was a significant increase from the preceding 5 years where 495-670 tonnes were caught annually, and was due to a number of domestic longliners that switched from targeting broadbill swordfish (shallow setting) to albacore tuna (deep setting) in northern parts of the fishery. In 2007, the higher catch was maintained (1,820t), and the targeted "deep setting" fishing expanded from the initial northern grounds as far south as the mid-NSW coast.

This significant shift in targeting prompted an immediate management response to limit the potential for increased capital investment in the fishery, given the uncertainty in the stock structure and productivity of the albacore stock in the SW Pacific and the recent policy initiative from the Australian Government to increase the economic return of Commonwealth fisheries to the Australian community (McDonald 2005). This response included the implementation of spatial management measures in the northern area of the ETBF (the "Albacore box") which effectively allowed targeted albacore fishing by existing participants and vessels in the fishery.

There is a need for a harvest strategy to manage the level of harvest in the ETBF. The current state of knowledge about albacore in Australian waters, however, was not sufficient to quantify its current state or productivity. The current state of knowledge about albacore in Australian waters, however, is not sufficient to quantify its productivity, nor connection with wider south Pacific stocks, so there is a pressing need for information on key biological parameters to support the implementation of the ETBF harvest strategy. This requires biological parameters such as age-at-maturity, growth rates, and mortality to determine target reference levels (Campbell et al., 2007). The Scientific Committee for the WCPFC also identified the need to improve our understanding of life-history parameters and stock assessments for albacore as high-priority. In particular, they noted that revised estimates of size/age compositions, sex ratios, growth parameters, maturity rates, longevity, and natural mortality were required.

2. Methods

2.1 Biological sampling

Gonads, sagittal otoliths and the first spiniform ray (fin spine) of the first dorsal fin were sampled from albacore caught in waters off Queensland and Tasmania in 2007 (Table 2.1). Sampling was port-based in Mooloolaba and Cairns, where the majority of albacore are landed. The Mooloolaba-based boats sampled operated in the 'albacore area' in the northern ETBF where albacore spawning was thought to occur (~15°S-25°S), while the Cairns-based boats operated inshore of the 'albacore area' targeting bigeye/yellowfin. Albacore were selected randomly during processing in the factories, measured to the nearest cm and weighed (whole and/or dressed) to the nearest g. Gonads, sagittal otoliths and fin spines were removed, labelled and sent to Hobart for processing either fresh or frozen.

Since albacore caught off Queensland are relatively large (10-25 kg), we also obtained samples from a number of smaller albacore caught by recreational fishers trolling off eastern Tasmania in March. One large recreational-caught albacore (20 kg) was also sampled from Tasmania in April. Fish were measured to the nearest cm and weighed (whole) to the nearest gram, and gonads, sagittal otoliths and fin spines were removed.

Table 2.1. Total number of measurements (length, weight, sex) and samples (gonads, otoliths, dorsal fin spines) collected for the project.

Month	Length	Weight (whole)	Weight (dressed)	Sex	Gonad	Otolith	Fin spine
Nov 06	1			1	1	1	
Dec 06	3			3	3		
Jan 07	36	36		36	36	23	33
Feb 07	24	24		22	22	20	21
Mar 07	26	25	1	26	26	26	26
Apr 07	20	20		20	20	20	18
May 07	-	-		-	-	-	-
Jun 07	89		50	74	39	38	74
Jul 07	33	19	12	30	27	21	17
Total	232	124	63	212	174	149	189

2.2 Length-weight relationship

The length-weight relationship is given as:

$$W = aL^b$$

where W is whole body weight in kg, L is fork length in cm, and *a* and *b* are parameters of the power function. Preliminary parameters were estimated using least-square linear regression of natural log transformed length and weight data

2.3 Sex ratio

The sex ratio of albacore was calculated, and chi-square tests were used to investigate the differences from an expected 1:1 for all fish, and by month and length class. Size frequencies of males and females were compared using a two-sample Kolmogorov-Smirnov test.

2.4 Direct ageing

Otoliths and fin spines were cleaned, dried and archiving into the 'CSIRO hardparts collection'. Albacore were identified for age estimation if both the otolith and spine had been collected. Of these, 97 fish were selected based on fork length, sex and month of sampling so that age could be estimated for the full size range sampled, both sexes and for each month sampled. An extra three otoliths and spines were selected based on fish size. Additional otolith and spines are stored in the archive for further age analysis if required in the future.

Otolith preparation and reading

To determine the most appropriate thickness to section the otoliths, 10 otoliths were initially selected and sent to the Central Ageing Facility (CAF) in Victoria for sectioning. The otoliths were embedded in clear casting polyester resin. For seven of the otoliths, four serial transverse sections were cut from each (one section including the primordium) and polished to 0.25 mm and 0.45 mm. The sections were mounted on glass slides with resin for viewing on a BX51 compound microscope. For the remaining three otoliths, were halved through the primordium to be viewed with oil immersion. After examining the otolith it was determined that 0.40 mm serial sectioning was the best for reading 'annual' increments in albacore otoliths.

The additional 90 otoliths selected for reading were sent to the CAF for serial sectioning at 0.40 mm thick. As already mentioned, otoliths were selected based on size of fish, sex, and month of sampling so that the full size and temporal range of fish sampled by sex was selected (Table 2.2). All sectioned otoliths were viewed on an Olympus BX51 compound microscope and the distance from the inflection point to the terminal edge of the otolith was measured along the external side of the otolith section (Fig. 2.1). Measurements were made using an image-analysis system; images were acquired with an Olympus F-view II digital camera mounted on the microscope connected to a PC computer. AnalySIS 3.2 (Soft Imaging System) was used to process the images and take measurements. For consistency, measurements were only made on sections that contained the primordium. The number of visible opaque growth zones was counted along the ventral 'long' arm of each otolith using the techniques developed for southern bluefin and bigeye tuna (see Clear et al., 2000; Anon., 2002) (see Fig. 2.1). A confidence score of 1 (poor) to 5 (excellent) was assigned to each reading. Otoliths were read two times by the same reader without reference to the previous reading, length of fish or date of capture. If the successive readings were in agreement, this estimate was used as the final increment count for the otolith. However, if the readings differed, a further reading was conducted with knowledge of the previous readings to decide on a final count. The final count was assigned an overall confidence based on the mean of the individual confidence scores. If no obvious pattern could be seen in the otolith section, a count was not made. The precision of readings (intra-reader consistency) was assessed using coefficients of variation (CVs) (Chang, 1982). The distance from the inflection point to the first three opaque growth zones (if present and clear) was measured along the external side of the otolith section. Edge type analysis (semi-direct validation; see review in Campana 2001) was attempted to determine if the timing of increment formation could be ascertained. To do this, the terminal edge of each otolith section was scored on the presence of an opaque or translucent zone.

Table 2.2. Number of otoliths and fin spines selected for age estimation by sex and length class.

Length	*Female*		*Male*		*Unknown*	
class (cm)	Otolith	Spine	Otolith	Spine	Otolith	Spine
45	2	2	-	-	4	4
50	1	1	2	2	8	8
60	-	-	1	1	-	-
70	4	4	3	3	-	-
75	-	-	-	-	-	-
80	-	-	-	-	-	-
85	9	9	7	7	-	-
90	14	12	10	11	-	-
95	3	3	17	18	-	-
100	1	1	12	12	-	-
105	-	-	2	2	-	-
Total	*34*	*32*	*54*	*56*	*12*	*12*

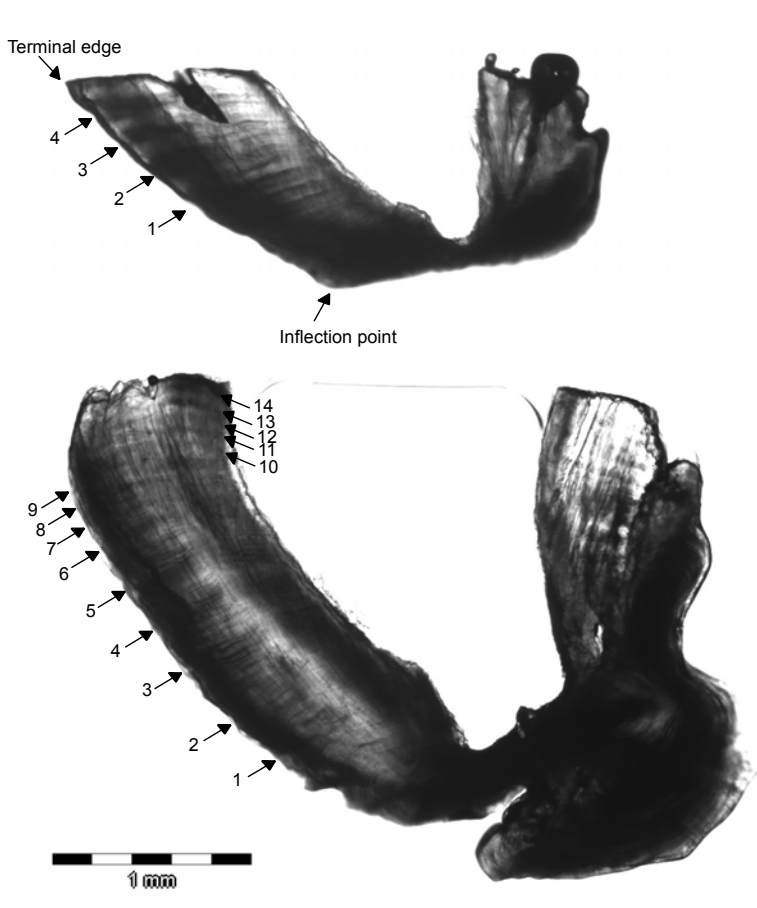

Figure 2.1. Transverse sections of otoliths from a 87 cm FL albacore (top) and a 103 cm FL (bottom) albacore. The otoliths show clear 'annual' opaque growth zones marked with arrows.

Fin spine preparation and reading

To determine the most appropriate position and thickness to section the fin spines for viewing growth increments, 10 fin spines were initially selected and sent to the CAF for sectioning. One of the fin spines was chosen to examine the appearance of increments in transverse sections cut along the length of the spine. The first section was cut 1 mm above the base of the spine through the condyle. Subsequent sections were cut along the spine 0.25 cm above the previous section. Thick (0.8 mm) and thin (0.25 mm) sections at each position were cut to determine the optimum thickness for viewing growth increments and the condyle radius was used as a reference for the positions of the sections along the spine. After examining these sections it was determined that sections in and near the condyle were not useful for viewing growth increments so the remaining nine fin spines were sectioned starting at a position equivalent to the condyle radius/2. A thick and a thin section were cut at condyle radius/2, condyle radius, then 0.5 cm further along the spine, then every 1 cm along the spine.
The fin spines were embedded in clear casting polyester resin and mounted on glass slides with resin for viewing on a compound microscope at a low magnification. After examining the fin spines it was determined that 0.5 mm sectioning was the best thickness for viewing growth increments in albacore fin spines. The position along the spine chosen to begin sectioning was the distance from the spine base equal to the condyle radius. Sometimes during sampling the condyle is damaged or not removed with the rest of the spine so another reference point that did not refer to the condyle was devised to identify the sectioning position for the remaining ninety samples. Following Rodriguez-Marin et al. (2007) sections were cut

at d/2, where d is the diameter of the spine above the “hollows” (Fig. 2.2). Several serial cross sections 0.5 mm thick were made from this position.

Most fin spines were dried at room temperature prior to sectioning but a subset of spines were kept frozen prior to sectioning to determine if one method produced clearer sections.
The fin spine sections were viewed on an Olympus BX51 compound microscope. The diameter of the sections and translucent zone diameters were measured (Fig. 2.3). For consistency, measurements were made on the first sections from each spine, i.e. those cut at the d/2 reference point, although subsequent sections were used to make counts. The number of translucent bands observed was counted (see Fig. 2.3) and used as the basis for age estimates. In an attempt to account for inner translucent zones in larger fish that may have been obscured by resorption or extra vascularisation, the diameter of translucent bands in younger fish was used to assign an age to the first observed translucent band in sections from larger fish. This “assigned age” of the first translucent band observed was added to the number of subsequent translucent bands observed to obtain a total count of translucent bands, which was recorded as the age estimate.

Fin spines were read three to four times by the same reader without reference to the previous readings, length of fish or date of capture. If the successive readings were in agreement, this estimate was used as the final age estimate for the fish. However, if the readings differed, a further reading was conducted with knowledge of the previous readings to decide on a final age estimate. A confidence score of 1 (poor) to 5 (excellent) was assigned to each count. The final age estimate was assigned an overall confidence based on the mean of the individual confidence scores. If no obvious pattern could be seen in the fin spine section, an age estimate was not recorded. The precision of readings was not assessed because the sets of readings were used to develop the reading protocols, rather than for otoliths where the methodology was already developed through previous experience reading other tuna otoliths (ie southern bluefin and bigeye tuna).

As for the otoliths, edge type analysis was attempted to determine any annual pattern of growth increment formation, i.e. if the timing of translucent band formation could be ascertained. The margin of each fin spine section was scored on the presence of an opaque or translucent zone.

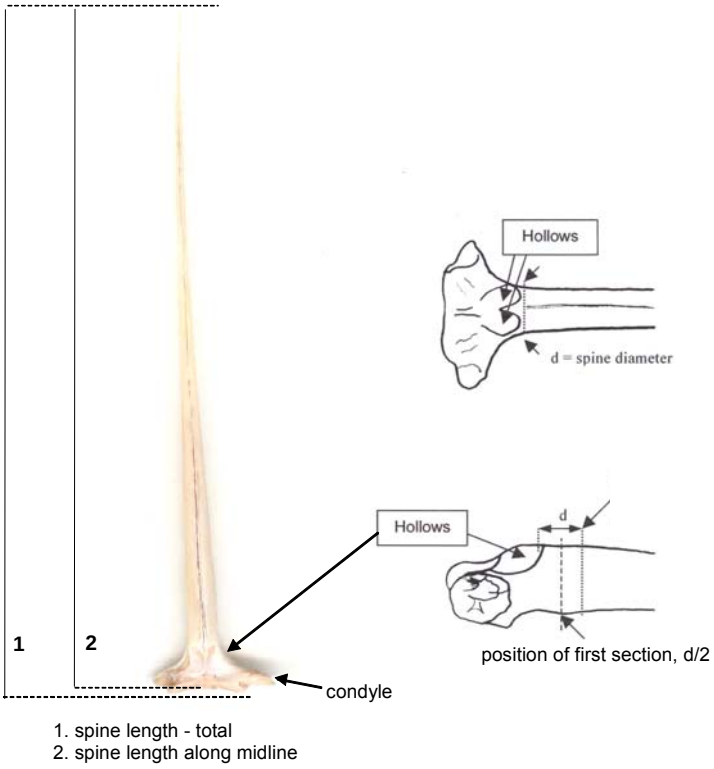

Figure 2.2. Albacore fin spine: spine length measurements (left); spine diameter measurement (top right) and the position where sections were cut (bottom right).

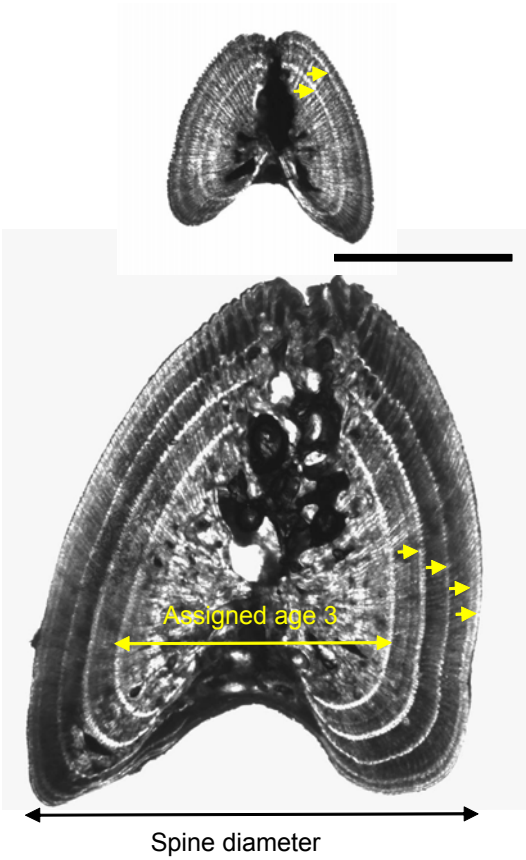

Fig. 2.3. Transverse sections of fin spines from a 49 cm FL albacore (top) and a 90 cm FL albacore (bottom). Translucent bands are marked with arrows. Ages estimated as 1 (top) and 7 (bottom). Note the 1-year-old fish has 2 translucent bands within the first annual increment and in the spine of the 7-year old the first 2 increments are obscured. Scale bar: 2 mm.

Comparison of structures

Comparisons were made between the ages estimated from otoliths and fin spines from the same fish to detect error in age determinations from the different structures. Age estimates were compared using an age-frequency table. Bias in age estimates was assessed to detect systematic disagreement between the structures using bias plots, in which age estimates from one structure were plotted as a function of the age estimates from the other structure (Campana et al., 1995). The level of difference in age estimates between structures was assessed using coefficients of variation (CVs) (Chang, 1982).

Growth

The von Bertalanffy growth function was fitted to the length-at-age data using the equation:

$$L_t = L_\infty (1 - e^{-k(t-t0)})$$

where L_t is the fork length (cm) at age t, L_∞ is the theoretical maximum fork length, k is the growth parameter (per year) and t_o is the theoretical age (years) at zero length. The equation was fitted using the non-linear regression function. Growth parameters were estimated using the least square method and the preliminary growth functions were compared using a modified analysis of the residual sum of squares (ARSS; Chen et al. 1992).

2.5 Reproduction

Gonad preparation, preservation & histological processing

All gonad collected were thawed if necessary, trimmed of fat, blotted dry and weighed to the nearest 0·1 g. If it was possible to distinguish the left and right gonads, they were weighed separately. In some cases, only one gonad (or a piece of gonad tissue) was obtained so total gonad weight was not determined. Each ovary was staged by the most advanced group of oocytes (MAGO) present in the ovary via a stereo microscope. The presence of residual hydrated oocytes (those left in the ovary after a spawning event) was noted for each ovary. The presence/absence of spermatozoa in the sperm duct was noted for males.
Gonad index was calculated as:

$$GI = W/L^3 \text{ X } 10^4$$

where W = gonad weight in g; and L = fork length in cm (Ramon and Bailey, 1996). Otsu and Uchida (1959) found no significant difference in the mean diameter of the MAGO, or in oocyte size frequency distributions, between left and right ovaries or along the length of the ovary. However, they did find a significant difference in mean diameter of the MAGO between the periphery and centre of the ovary suggesting that oocyte development can vary across the ovary. Consequently, a core subsample (lumen to the periphery) was taken from the mid-section of either the left or right ovary and fixed in 10% buffered formalin for histological sectioning. Subsamples from the mid-section of testes were also taken and fixed in 10% buffered formalin. Samples that were selected for histological analysis (detailed below) were embedded in paraffin, and standard histological sections prepared (cut to 6 µm and stained with Harris' haematoxylin and eosin). During preparation, each gonad sample was positioned so that a cross-section was cut from the core to the periphery. Histological sections were viewed (and classified) on an Olympus BX51 compound microscope. Digital images of the histological sections were acquired with a Zeiss Axiocam HRC colour digital camera mounted on an Axioplan compound microscope connected to a PC computer. Axiovision (Soft Imaging System) was used to process the images.

Histological classification of females

Histological sections were prepared for all albacore classified as female. A comparison of histological sections prepared from fresh and frozen-thawed material showed that frozen tissue, although not perfect due to the rupture of some cells, were nevertheless suitable for staging ovaries as the oocytes, postovulatory follicles and all stages of atresia could be identified and classified. It is recommended, however, that the gonad material be thawed and preserved as soon as possible after sampling to reduce the possibility of tissue deterioration while frozen. Histological sections were classified with criteria similar to those developed for skipjack tuna, *Katsuwonus pelamis* (Hunter et al., 1986), yellowfin tuna, *Thunnus albacres* (Schaefer, 1996; 1998) and southern bluefin tuna, *Thunnus maccoyii*, (Farley and Davis, 1998):

Oocytes:

Each ovary was staged by the most advanced group of oocytes (MAGO) present into one of 5 classes (Fig. 2.4) and the mean diameter (random axis) of 10 oocytes from the MAGO was measured to the nearest μm:

1. Unyolked (previtellogenic): ~ <150 μm in diameter. Cytoplasm strongly basophilic (dark purple stained) (Fig. 2.4A)
2. Early yolked: ~150-250 μm in diameter. Distinct thecal layer and zona radiata present. (Fig. 2.4B)
3. Advanced yolked: ~350- 500 μm in diameter. Small pinkish-red (acidophilic) yolk globules appear in outer regions of the oocyte. The zona radiata is thick and striated. (Fig. 2.4B)
4. Migratory nucleus: ~400-500 μm in diameter. The nucleus migrates to the periphery of the oocyte and oil droplets coalescence. The nucleus breaks down when it reaches the periphery, the yolk globules coalesce into yolk plates. (Fig. 2.4C)
5. Hydrated: > 500 μm in diameter. The oocyte rapidly increases in size as hydration occurs and becomes pale pink in colour. The zona radiata becomes thinner. (Fig. 2.4D)

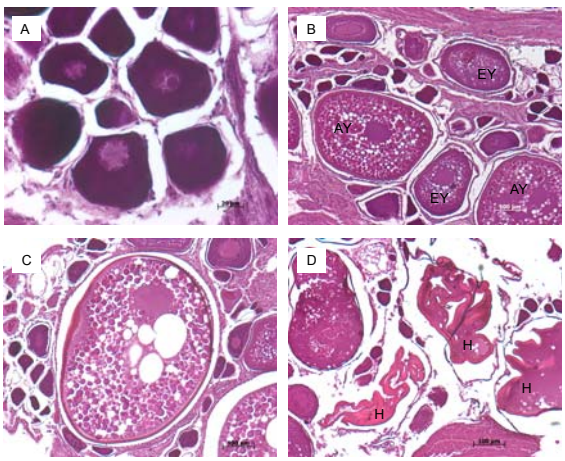

Figure 2.4. Oocyte development stages in albacore tuna ovaries. (A) Unyolked oocytes. (B) Early yolked (EY) and advanced yolked (AY) oocytes. (C) Migratory nucleus oocyte. (E) Hydrated oocytes (H).

Postovulatory follicles:

The presence/absence of postovulatory follicles was also recorded. It is difficult to estimate the age of these postovulatory follicles because the rate of degeneration is unknown. However, skipjack tuna, yellowfin tuna and bigeye tuna, *Thunnus obesus*, are known to resorb their postovulatory follicles within 24 hours of spawning, and all spawn in water temperatures above 24°C (McPherson, 1988; Nikaido et al., 1991; Schaefer, 1996). Since albacore also

spawn in these temperatures (Ueyanagi, 1969) and water temperature appears to be the dominant factor determining resorption rates (Fitzhugh and Hettler, 1995), we aged postovulatory follicles using the criteria developed by McPherson (1988), Nikaido et al. (1991) and Schaefer (1996). Postovulatory follicles were classified as either absent, new (0 hours old), less than 12 hours old, or greater than 24 hours old (Fig. 2.5).

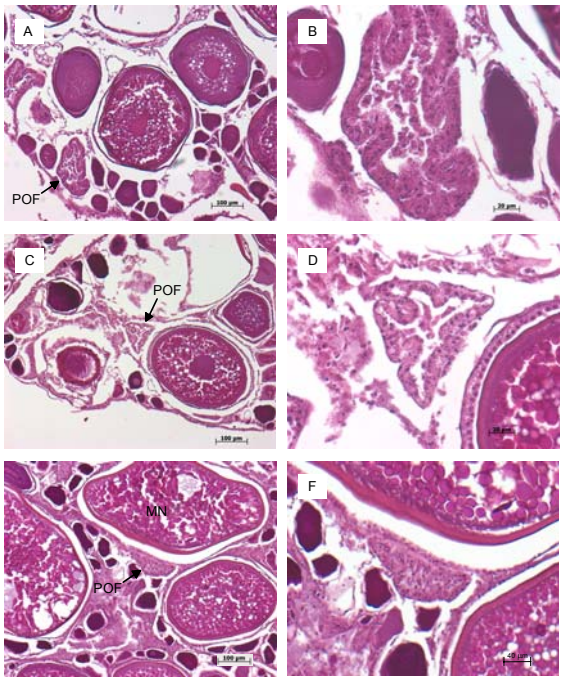

Figure 2.5. Postovulatory follicle stages (POF) in albacore ovaries. Photos on the right are higher magnification photos of the POF marked on the left. (A - D) Less than 12 hours old. (E and F) Greater than 12 hours old.

Oocyte atresia (resorption):

Finally, ovaries were classified by the level of atresia of fully yolked oocytes present. During the alpha (α) stage of atresia, the zona radiata disintegrates, the granulose cells invade the oocyte and yolk resorption takes place (Fig 2.6A & B). During the beta (β) stage of atresia, the remaining granulosa and thecal cells are reorganised and resorbed leaving a compact structure containing several intercellular vacuoles (Fig. 2.6C). Gamma (γ) stage atresia is smaller than β atresia and contains light-yellow (stained) material. Delta (δ) atresia is very small, composed of granulosa cells which stain a distinctive dark-yellow (orange) and are located within the ovarian connective tissue (Fig 2.6D). Delta atresia was specifically identified using criteria given in Hunter and Macewicz (1985b) and McDonough et al. (2003). The level of α atresia in the ovary was categorised as: none, <10%, 10-50%, >50%, and 100%. The presence or absence of beta, gamma and delta atresia was also recorded.

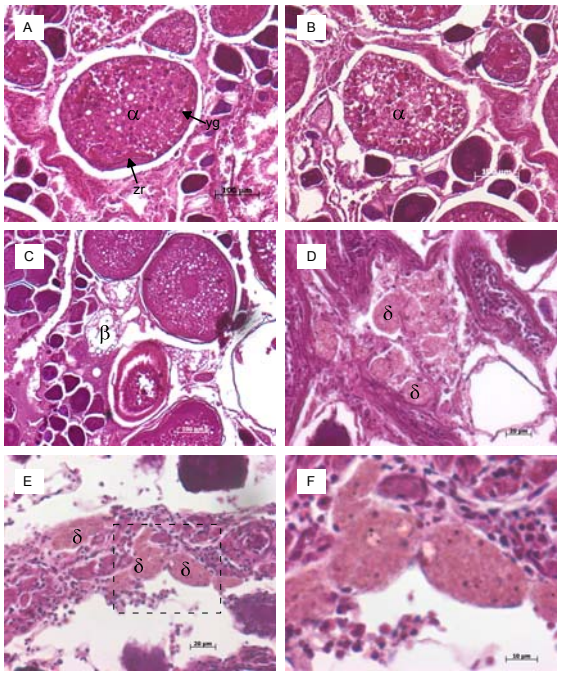

Figure 2.6. Stages of atresia of yolked oocytes in albacore tuna ovaries. (A) early alpha stage of atresia. (B) late alpha stage of atresia. (C) beta stage of atresia. (D-F) delta stage of atresia. (F) is the inset shown in (E) at a higher magnification. zr = zona radiata, yg = yolk granule.

Females were then classified into one of 6 reproductive states depending on the oocytes, atretic state and postovulatory follicle class present in histological sections adapted from the schemes developed by Schaefer (1998), Farley and Davis (1998) and more recently by Brown-Peterson et al. (In prep). Females in stages 3-6 are classed as mature.

(1) Immature: Ovaries contains unyolked oocytes only which are densely packed. No partially yolked, advanced yolked, or advanced yolked oocytes in any stage of atresia (including delta atresia). No residual hydrated oocytes present.

(2) Developing: Ovary contains early yolked oocytes as the most advanced state. No atresia of advance yolked oocytes (in any stage) or post ovulatory follicles present.

(3) Spawning capable (active but non-spawning): Ovary contains advanced yolked oocytes but no evidence of spawning activity (migratory nucleus oocytes, hydrated oocytes or postovulatory follicles). Less than 50% of advanced yolked oocytes are in the alpha stage of atresia.

(4) Actively spawning: Ovary contains advanced yolked oocytes and evidence of spawning activity (migratory nucleus or hydrated oocytes or postovulatory follicles). Less than 50% of advanced yolked oocytes are in the alpha stage of atresia.

(5) Regressing (Atretic): Ovaries contain either:

(i) >50% of advanced yolked oocytes in the alpha stage of atresia (major atresia but potentially reproductive);

(ii) 100% of advanced yolked oocytes in the alpha stage of atresia (early post-spawning),

(iii) no yolked oocytes are present but oocytes in the beta stage of atresia are (advanced post-spawning).

(6) Regenerating: Ovaries contain no advanced yolked oocytes. However, advanced yolked oocytes in the gamma and/or delta stage of atresia are present.

Histological classification of males

Although females are generally given priority in reproductive studies, 25 males were selected for histological analysis, based on fish size and month of sampling to examine development stages and confirm the sex of small fish.

Albacore testes are considered to be an 'unrestricted spermatogonian tesis-type' as spermatogonia are found along the length of the tubules (Grier, 1981). Males are more difficult to classify than females because there is a gradual change in the relative proportion of spermatocytes, spermatids and spermatozoa in the testes, rather than distinct reproductive stages as for females. The classification of males was based on the most advanced sperm maturation stage present in the lobules: spermatogonia, spermatocytes, spermatids or spermatozoa (Fig. 2.7). When the lumen of the testis was visible, evidence of imminent or recent spawning was also noted by presence/absence of spermatozoa and the shape of the sperm duct (Fig 2.7). Males were considered mature if spermatozoa were present in the sperm duct (Schaefer, 2001).

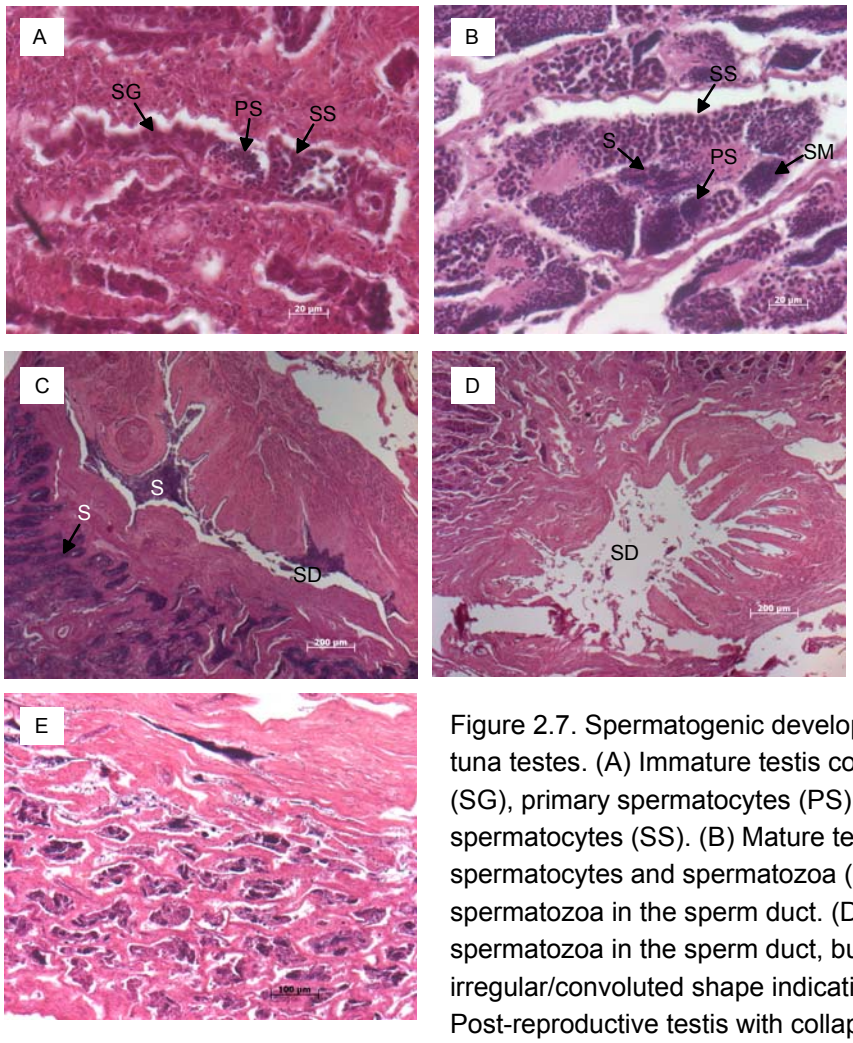

Figure 2.7. Spermatogenic development stages in albacore tuna testes. (A) Immature testis containing spermatogonia (SG), primary spermatocytes (PS) and secondary spermatocytes (SS). (B) Mature testis contining spermatogonia, spermatocytes and spermatozoa (S). (C) Mature testis with spermatozoa in the sperm duct. (D) Mature testis with no spermatozoa in the sperm duct, but the duct has an irregular/convoluted shape indicating recent spawning. (E) Post-reproductive testis with collapsed lobules.

Size at 50% maturity

Using the histological classification scheme described above, the criteria to distinguish mature from immature females is based on the presence of advanced yolked oocytes or atresia of advanced yolked oocytes (alpha to delta) as evidence of past reproductive activity. To determine if the maturity criteria was suitable to estimate of length at 50% maturity, a logistic

regression was fitted to our preliminary data set for females sampled in January to July (i.e. December samples were not included):

$$P(maturity \mid L) = (\exp(a+bL)) / (1+\exp(a+bL))$$

where P is the estimated proportion of mature individuals at fork length L, and a and b are parameters that define the shape and position of the fitted curve. The predicted length at 50% maturity (L_{50}) was calculated as:

$$L_{50} = -a/b$$

Spawning frequency

Spawning frequency of females was estimated by the postovulatory follicle method of Hunter and Macewicz (1985a). This method uses the incidence of mature females with postovulatory follicles less than 24 hours old to define the fraction of the population spawning. Only samples collected during January and February (when actively spawning females were sampled were included.

Fecundity

To determine fecundity type (determinate or indeterminate), we measured the diameters of oocytes in the ovary of an albacore with early hydrated oocytes. A core subsample of 0.182 g was taken from the mid-section of one ovary lobe, weighed to the nearest 0.01 mg and fixed in 10% buffered formalin. The ovary sample was teased apart and each oocyte (greater than 150 μm in diameter) was measured in a random direction to the nearest 0.001 mm. Approximately 1,000 oocytes were viewed and measured using an image-analysis system; images were acquired with an Olympus F-view II digital camera mounted on a Wild M5a stereomicroscope connected to a PC computer. AnalySIS 3.2 (Soft Imaging System) was used to process and enhance the images and measure oocyte diameters.

Of the ovaries sampled during the project, 14 contained migratory nucleus (MN) or hydrated (Hyd) oocytes that indicated that it may be possible to estimate batch fecundity. A core subsample (periphery to the lumen) of between 0.06-0.08 g was taken from both sides of each ovary lobe, weighed to the nearest 0.01 mg and fixed in 10% buffered formalin for potential fecundity estimates. Of these, 9 ovaries were identified as suitable for estimating batch fecundity as the batch of oocytes to be spawned could be clearly separated from the advanced yolked oocytes and new postovulatory follicles were not present. Batch fecundity was estimated by the gravimetric method for 7 of these ovaries (Hunter et al., 1985). Each subsample was teased apart to separate out the hydrated or migratory nucleus oocytes, which were counted under a Wild M5a stereomicroscope. The number of hydrated/migratory nucleus oocytes per gram of ovary was raised to the weight of both ovaries to give an estimate of batch fecundity for each of the four subsamples. The mean of these estimates gave the batch fecundity estimate for the fish.

3. Results and Discussion

3.1 Length composition

The size of albacore sampled in Mooloolaba, Cairns and Tasmania ranged from 48-108 cm fork length, and 2.5-25.6 kg whole weight. Large albacore dominated the catches off Queensland (79-108 cm) (Fig. 3.1). This is not surprising given that fish of these sizes are

typical of longline catches in the South Pacific. Small fish dominated the catch off Tasmania (48-74 cm) although two large (>80 cm FL) fish were also sampled (Fig. 3.1). Although the sample size was small, there were two modes in the size frequency of albacore sampled; one at 48-52 cm and another at 70-74 cm. These most likely correspond to the first and third modes in the length frequency of albacore caught in the New Zealand troll fishery (Griggs, 2005) which have been suggested to correspond to cohorts (1-3 year olds) recruiting to the troll fishery. The large (102 cm FL) female sampled off Tasmania was unusual given that very few females of this size were found in the Queensland samples.

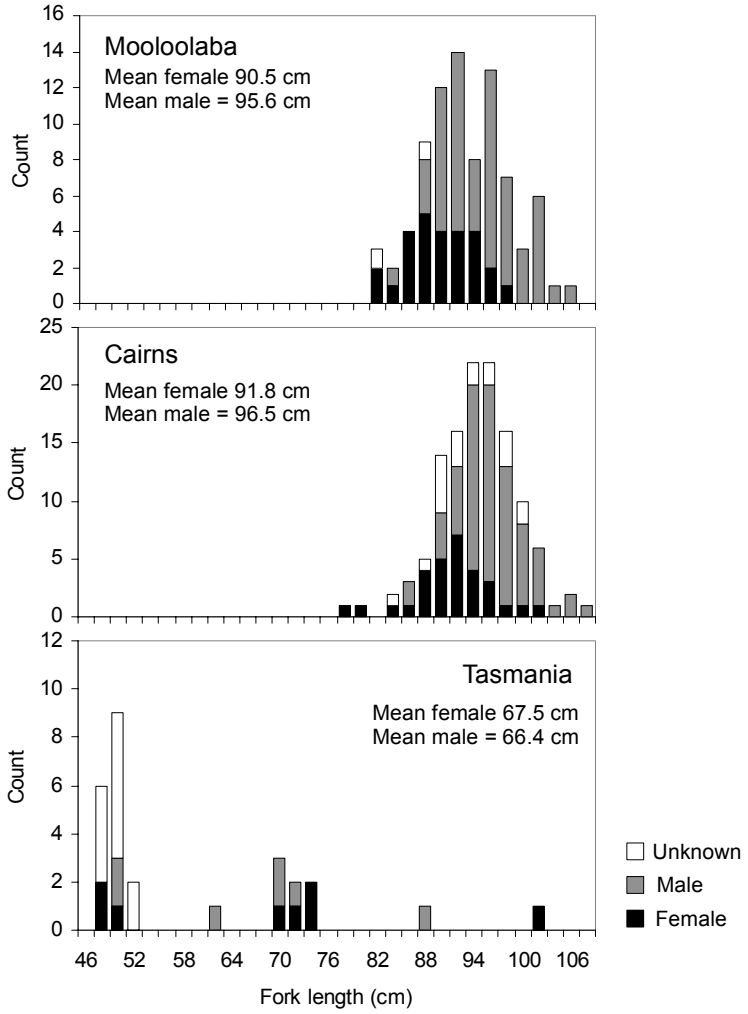

Figure 3.1. Length frequency (2 cm intervals) distributions by sex for albacore sampled in Mooloolaba, Cairns and Tasmania. Mean length by sex is given.

3.2 Length-weight relationship

Figure 3.2 shows the relationship between fork length and weight (dressed and whole). Preliminary length-weight parameters were estimated as:

$$W = 1.24 \times 10^{-5} .L^{3.1211}$$

$$r^2 = 0.9938$$

Although sample size is small, analysis of covariance found no significant difference in the length–dressed weight relationship between sexes ($F = 1.27$, $P = 0.26$).

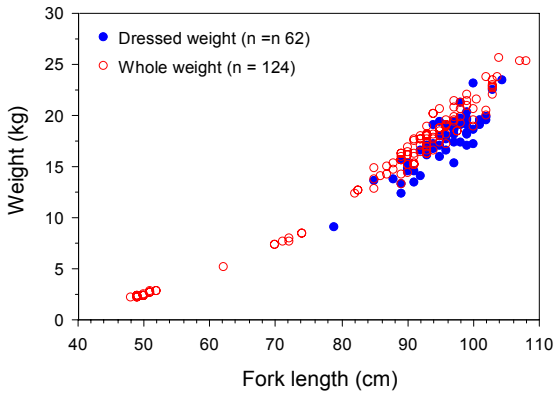

Figure 3.2. Plot of fork length (cm) to weight (kg) for albacore sampled in Mooloolaba, Cairns and Tasmania. Note that weight data for Cairns is predominantly dressed weight (gilled and gutted), and for Mooloolaba and Tasmania is whole weight. n=187.

3.3 Sex ratio

The sex ratio of fish sampled off Queenland was significantly different from the expected 1:1 in both Mooloolaba ($\chi^2 = 17.95$, $P < 0.001$; sex ratio = 2.4:1) and Cairns ($\chi^2 = 9.00$, $P = 0.003$; sex ratio = 2.0:1) with males dominating both catches. A Kolmogorov-Smirnov test detected significant differences ($P < 0.001$) in length frequency distributions between males and females (Fig. 3.3) with more males in length classes ≥ 94 cm FL, but more females length classes 84-86 cm FL. Consequently, the mean size of males sampled was larger than for females. Of the fish <90 cm FL sampled off eastern Tasmania, sex was identified for only 14 as the gonads of many fish were extremely small (often only a few grams) and histological sections did not always reveal the necessary detail to determine sex. The sex ratio of these fish was 1:1.

A dominance of males in longline catches of fish > ~90 cm FL has been reported in other studies from the Pacific, Atlantic, and Indian Oceans (Ramon & Bailey, 1996; Ortiz de Zárate et al., 2005; Wu and Kuo, 1993; Hsu and Chen, 2005). It has been suggested that the dominance of large males is possibly due to both differential mortality of sexes and differential growth rate after maturity (Collette and Nauen, 1983). A preponderance of males in larger size classes has also been reported for yellowfin, bigeye, Atlantic bluefin, and southern bluefin tuna (Clay, 1991; Nikaido *et al.*, 1991; Sun et al., 2005; Farley et al., 2007). Megalofonou (1990), however, found that the in the Agean Sea, sex ratio favoured males (2.1:1) for small albacore 54-89 cm FL. The sex ratio was approximately 1:1 up to the 67 cm length class, after which males dominated. Given that the size at first maturity is lower for Mediterranean albacore (62 cm; Arena, 1980) it is not surprising that males start to dominate the catch at a smaller size than in other regions, if differential growth occurs after maturity. A sex ratio of 1:1 for juveniles has also been reported for the New Zealand troll fishery (47-81 cm size fish) (Griggs & Murray 2000) and for fisheries in the North Pacific (Bartoo and Foreman, 1994).

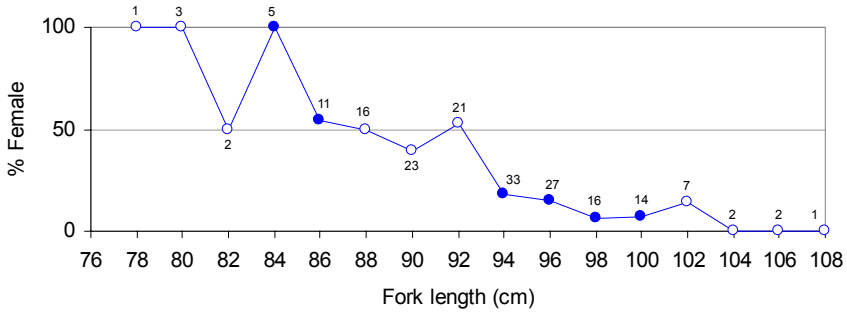

Figure 3.3. Percent female by 2-cm length for albacore sampled off Queensland. Sample sizes are shown. ● indicate significant χ^2 tests ($P < 0.05$)

3.4 Direct ageing

Estimating age using otoliths

The clarity of 'annual' increments in albacore otoliths varied between fish. Of the 97 otoliths prepared, 14 were considered unreadable and not given a final age estimate and thus were excluded from subsequent analyses. One additional otolith had a mean confidence score of 1 and was also excluded. The CV between blind readings of the remaining 83 otoliths was 5.79%. When successive readings of otoliths differed, 73% were by ±1 year, indicating a reasonable level of precision.

Assuming increments form annually in albacore otoliths (see below), age estimates for our subsample of albacore ranged from 1+ to 9+ years for females and 1+ to 14+ for males. The 13+ and 14+ males had fork lengths of 106 and 104 cm respectively. This estimate of longevity is similar to that obtained by Labelle et al. (1993) based on vertebral-ring counts of 13 years for a 107 cm fish. Length at age differed between individuals, suggesting that growth is variable in albacore (Figure 3.4). For example, fish in the 90 cm length class (90–94.9 cm) ranged in age from 5+ to 9+ years. Although our sample size is small, we calculated mean lengths-at-age and von Bertalanffy growth parameters by sex (Table 3.1 and 3.2). Significant differences were not detected in mean lengths-at-age between males and females (unpaired t-tests by age class; $P>0.05$) although males were larger on average than females after age 4, and the t-test P-values declined with increasing age (e.g. at age 4, $P = 0.293$ and by age 7, $P = 0.073$). Significant differences were detected in Bertalanffy growth parameters between the sexes (ARSS; $F = 4.84$; d.f. 3, 67, $P = 0.004$). The difference in L_∞ between males and females (104.5 and 98.4 cm respectively) is consistent with the size frequency data collected for the project, where only 1.8% of females were >98 cm FL while 30.0% of males were greater than this size (also see Fig. 3.1).

Semi-direct age validation using edge type analysis was not possible in the current project. This type of validation requires monthly sampling of specific age classes to determine the timing of opaque growth zones increment formation. While monthly sampling of juveniles is not possible in the ETBF, it should be possible for older fish (but was not achieved in the current project). In addition to the lack of monthly samples, the terminal edge of many otolith sections was not clear enough to be sure of the terminal edge type.

Despite the current lack of age validation for annual increments in otoliths, researchers at SPC in Noumea have recently compared presumed daily increment counts (validated for 50-100 cm North Pacific albacore; Laurs et al., 1985) with the position of presumed annual

increments in sectioned otoliths, similarly to that undertaken for bigeye tuna (Farley, et al., 2006). SPC's work identified the region of the first and second annual increments in otoliths based on the positions of the 365th and 730th daily increments (Kerandel, et al., 2006). A comparison of the distance from the first inflection point to the first opaque growth zones in our otoliths with the mean distance to the 365th and 730th daily increment (Fig. 3.5) suggests that the first two annual increments are being successfully identified in our sectioned otoliths. It also shows that that the first and second annual opaque growth zones are deposited before the first/second birthdays. Since albacore spawn during summer, it appears that the first opaque growth zone is deposited during the juvenile's migration from spawning (tropical in summer) to feeding latitudes (temperate the next summer), and the second opaque growth zone is deposited the following winter.

Although it appears that the third annual increment is not being successfully identified (because on average it occurred around the 730th daily increment; Fig. 3.5), Kerandel, et al.'s (2006) work suggests that daily increment counts underestimated annual age after age ~2 years (see their Fig. 6). Thus we think that increments 1-3 are being identified correctly in our study. In addition, estimates of length at age 1 and 2 years of 45-50 cm and 70 cm respectively from daily age estimation, are consistent with our estimates of mean length at age 1+ and 2+. Confirming the position of these increments is important, as this part of the otolith is often the most difficult to read.

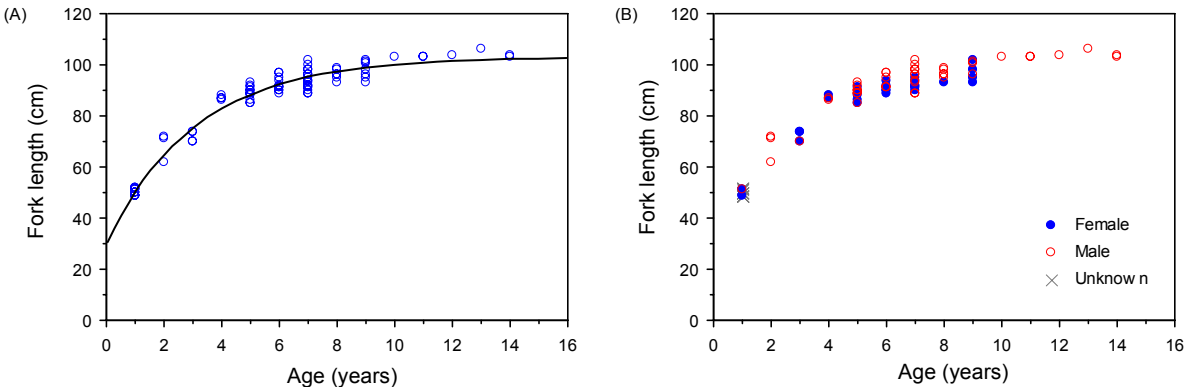

Fig. 3.4. Length at age estimates for albacore tuna based on otolith readings. (A) All fish with a fitted von Bertalanffy growth curve. (B) By sex.

Table 3.1. Mean observed length-at- age and predicted length-at-age from von Bertalanffy models (see Table 3.2) for albacore based on otolith readings.

Age	*Observed*			*Von Bertalanffy model*		
	Female	*Male*	*All*	*Female*	*Male*	*All*
0+	-	-	-	27.6	35.2	30.8
1+	50.0 (2)	51.0 (1)	50.2 (13)	49.6	53.3	50.6
2+	-	68.3 (3)	68.3 (3)	64.8	66.6	64.9
3+	72.7 (3)	70.0 (1)	72.0 (4)	75.3	76.5	75.3
4+	87.5 (2)	86.5 (2)	87.0 (4)	82.5	83.8	82.9
5+	88.4 (5)	89.3 (6)	88.9 (11)	87.4	89.2	88.4
6+	91.0 (5)	93.7 (6)	92.5 (11)	90.8	93.2	92.4
7+	93.0 (7)	94.9 (10)	94.1 (17)	93.2	96.1	95.2
8+	-	96.2 (6)	96.2 (6)	94.8	98.3	97.3
9+	97.0 (4)	99.2 (3)	97.9 (7)	95.9	99.9	98.9
10+	-	103.0 (1)	103.0 (1)	96.7	101.1	100.0
11+	-	103.0 (2)	103.0 (2)	97.2	102.0	100.8
12+	-	103.8 (1)	103.8 (1)	97.6	102.6	101.3
13+	-	104.5 (2)	104.5 (2)	97.8	103.1	101.8
14+	-	104.0 (1)	104.0 (1)	98.0	103.5	102.1

Table 3.2. Preliminary otolith-based von Bertalanffy growth parameters for albacore by sex. (NB 10 juveniles had unknown sex.)

Sex	*Parameter*	*Estimate*	*S.E*	*Lower 95% CI*	*Upper 95% CI*
All	L_∞	102.865	1.199	100.5	105.252
(n=83)	k	0.321	0.022	0.278	0.364
	t_o	-1.107	0.139	-1.384	-0.830
Males	L_∞	104.494	1.599	101.267	107.720
(n=45)	k	0.302	0.032	0.238	0.366
	t_o	-1.362	0.328	-2.025	-0.699
Females	L_∞	98.379	1.818	94.635	102.124
(n=28)	k	0.373	0.045	0.281	0.465
	t_o	-0.883	0.253	-1.405	-0.361

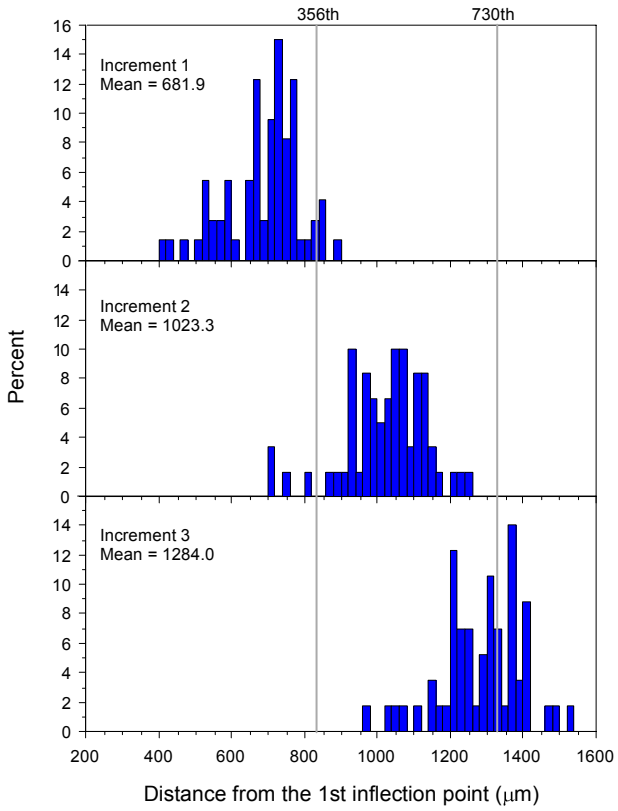

Fig. 3.5. Histograms of the distance from the first inflection point to the first, second and third opaque growth zone on otoliths. Grey vertical lines represent the mean distance to the 356th and 730th daily increment measured by scientists at the Secretariat of the Pacific Community (New Caledonia) on albacore otoliths sampled in New Caledonia and New Zealand (see Kerandel et al., 2006).

Estimating age using dorsal spines

In the cross sections of fin spines two types of bands, translucent and opaque, formed growth increments that were visible under both transmitted and reflected light. Translucent bands were generally narrower than opaque bands but obvious in different forms: fine or thick single rings, and double or triple translucent rings that were separated by narrow opaque areas. A single translucent band and adjacent opaque band were assumed to represent one year of growth. However double or triple translucent bands were common. Multiple bands were considered to belong to the same "annual" growth increment if:

1. the distance between them was less than the distance to the preceding and subsequent translucent band;
2. the translucent bands converged at the vertex of the spine.

Despite the low number of samples, we did attempt to establish and adhere to these protocols for interpreting and counting growth increments in fin spines. In cases where double and triple bands are not recognized, but counted as individual increments, age will be overestimated. Therefore validation of the methods is critical to ensure the accuracy of these protocols.

In the fin spines of albacore around 50 cm FL (the smallest fish sampled), the core was not obscured by vascularisation and resorption and, in all samples, two translucent bands were

present (Fig. 2.3). One interpretation is that 50 cm albacore are age 2+. However, the two translucent bands were separated only by a narrow opaque zone, so an alternative interpretation is that the first growth increment included a double translucent band and therefore 50 cm albacore were age 1+. The alternative was accepted for this study, and was supported by results from daily increment studies of Pacific albacore that predicted mean length at age 1 to be 45-50cm and age 2 fish to be 60-70 cm (Bigelow et al. 1993, 1995; Kerandel et al. 2006).

The clarity of growth increments in fin spines varied between fish. Of the 100 fin spines examined, 3 were considered unreadable and not given a final age estimate, and thus were excluded from subsequent analyses. Eleven out of the 100 fin spines examined were treated differently before sectioning. Eighty nine spines were allowed to dry before sectioning, 11 were kept frozen until just before sectioning. Rodríguez-Marín et al. (2007) noted that growth increments were clearer on spines that had been allowed to dry but we found no observable difference of clarity in the fin spines included in this study.

The core of the spine sections was obscured to some degree due to vascularisation and resorption in about 50% of fish larger than 71 cm FL and in 100% of fish larger than 87 cm FL. To determine how many inner translucent bands from these fish were obscured the table of fin spine band diameters was used to as an aid to determine the ”age” of the first visible translucent band (Table 3.3). There was only a small number of data to construct this key; more samples are required to improve the reliability of this information. Without accurate knowledge of the expected band diameters of the inner increments, age may be consistently underestimated.

Previous studies based on Atlantic and Mediterranean albacore found that at least the first translucent bands within an increment form annually between July and September (Cort 1991, Zarate et al. 1996, Megalofonou 2000, Santiago and Arrizabalaga 2005). However some increments comprise more than one translucent (and opaque) band per year and it is hypothesized that these coincide with intra-annual migrations and are caused by the large bioenergetic stress associated with migration (Bard and Compean-Jimenez 1980, Compean-Jimenez and Bard 1983).

A single translucent band is likely to be the result of normal annual environmental and physiological fluctuations but it is possible that any extra translucent bands are the result of an event such as migration that will expose fish to different environments and place them under extra physiological stress. Given that little is known about the migration patterns of albacore in the ETBF further research is required to identify if this is a primary cause of growth patterns in fin spines.

Table 3.3. Mean spine diameter (mm) for fish estimated to be 1+, 2+ and 3+, used to “assign an age” to the first observed translucent band in sections from larger fish.

Number of “annual” growth increments in fin spine section	1	2	3
Mean spine diameter (mm)	2.36	3.73	4.88
Number of samples	14	3	4

Assuming growth increments form annually in albacore fin spines, age estimates for our subsample of albacore ranged from 1+ to 10+ years for females and 1+ to 11+ for males. Length at age differed between individuals, suggesting that growth is variable in albacore (Figure 3.6). For example, fish in the 90 cm length class (90–94.9 cm) ranged in age from 5+ to 7+ years and fish in the 100 cm length class (100-104.9 cm) ranged in age from 8+ to 11+ years. Although our sample size is small, we calculated mean lengths-at-age and von Bertalanffy growth parameters by sex (Table 3.4 and 3.5). Similar to otolith results, significant differences were not detected in mean lengths-at-age between males and females (unpaired t-tests by age class; $P>0.05$), while significant differences were detected in Bertalanffy growth parameters between the sexes (ARSS; $F = 3.54$; d.f. 3, 79, $P = 0.018$). As explained in the previous section, age validation using edge type analysis was not possible due to the lack of monthly sampling of specific age classes. However, unlike otoliths, the terminal edge of fin spines, at least in younger fish, is clear enough to determine if the terminal edge is opaque or translucent. If more samples can be collected throughout the year, fin spines could be used to determine the timing of annual growth increment formation.

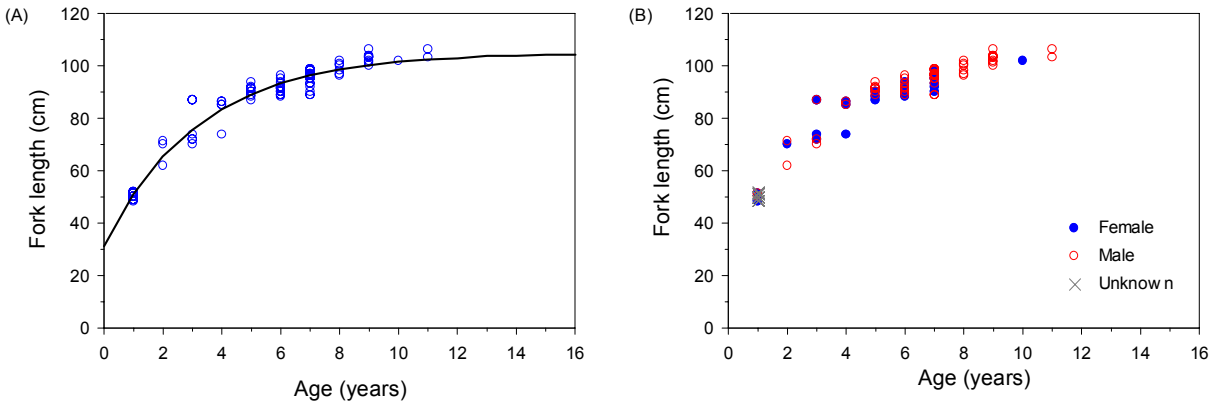

Fig. 3.6. Length at age estimates for albacore tuna based on fin spine readings. (A) All fish with a fitted von Bertalanffy growth curve. (B) By sex.

Table 3.4. Mean observed length-at- age and predicted length-at-age from von Bertalanffy models (see Table 3.5) for albacore based on spine readings.

Age	*Observed*			*Von Bertalanffy model*		
	Female	*Male*	*All*	*Female*	*Male*	*All*
0+	-	-	-	24.8	33.8	31.2
1+	59.3 (3)	50.5 (2)	50.1 (17)	50.4	51.9	50.7
2+	70.0 (1)	66.5 (2)	67.7 (3)	66.8	65.5	65.1
3+	80.0 (4)	76.3 (3)	78.4 (7)	77.4	75.8	75.6
4+	81.7 (3)	85.5 (2)	83.2 (5)	84.3	83.5	83.4
5+	88.3 (3)	91.2 (6)	90.2 (9)	88.7	89.3	89.0
6+	90.6 (11)	92.3 (7)	91.3 (18)	91.5	93.6	93.2
7+	93.8 (6)	95.5 (14)	95.0 (20)	93.4	96.9	96.3
8+	-	98.9 (6)	98.9 (6)	94.6	99.4	98.5
9+	-	102.9 (9)	102.9 (9)	95.3	101.2	100.2
10+	102.0 (1)	-	102.0 (1)	95.8	102.6	121.4
11+	-	104.5 (2)	104.5 (1)	96.1	103.6	102.3

Table 3.5. Preliminary spine-based von Bertalanffy growth parameters for albacore by sex. (NB 12 juveniles had unknown sex.)

Sex	*Parameter*	*Estimate*	*S.E*	*Lower 95% CI*	*Upper 95% CI*
All	L_∞	104.729	1.614	101.524	107.935
(n=97)	k	0.309	0.025	0.259	0.358
	t_o	-1.144	0.156	-1.454	-0.834
Males	L_∞	106.822	2.158	102.487	111.158
(n=53)	k	0.285	0.033	0.219	0.352
	t_o	-1.335	0.311	-1.960	-0.710
Females	L_∞	96.702	2.340	91.916	101.488
(n=32)	k	0.439	0.073	0.290	0.588
	t_o	-0.676	0.294	-1.277	-0.074

Comparison of structures

Age was estimated from otoliths and spines by different readers with no reference to each others counts. A direct comparison of age estimates from otoliths and fin spines could be made for 79 fish. The age estimate from fin spines agreed with the estimate from otoliths in 41% of cases and 81% were within 1 year of each other. For otolith ages 1-7 years, fin spine ages were within 1 year of the otolith ages for 90% of fish. The mean CV between age estimates was 9.13%, but was slightly lower (8.03) for ages 1-7 years. Comparisons of age estimates are shown in an age frequency table (Table 3.6) and an age-bias plot (Fig. 3.7A). These show no, or low, bias for ages 1-7 years. However a bias exists after this age; otolith-based age estimates are generally higher than the spine-based age estimates. Fig. 3.7B shows that the agreement rate between structures generally decreases with increasing age.

Table 3.6. Age frequency table summarising pairwise comparisons of age estimates from otoliths and fin spines (n = 79).

	Otolith age														
Spine age	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
1	13														13
2		2	1												3
3		1	2	2											5
4			1	1	3										5
5				1	2	2	2								7
6					5	5	5								15
7					1	2	5	4	4						16
8						1	1	1	1						4
9							2		1	1	1	1		2	8
10									1						1
11											1		1		2

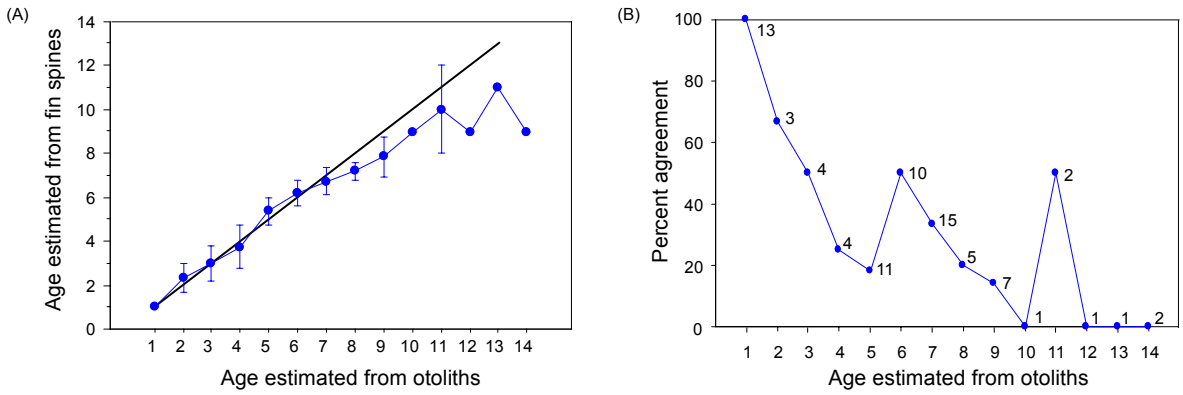

Fig 3.7. Comparisons of age estimates from otoliths and fin spines. (A) Age bias plot of paired age determinations (+/- 2 se) with the 1:1 (zero difference line) indicated. (B) Agreement rate between age estimates (sample sizes are indicated).

Different physiological pathways determine growth in otoliths and fin spines. Fin spines are skeletal material and are vascularised and as such can be subject to resorption that can cause the core area to become obscured. The first increments, deposited when the fish are young, are situated at the core of the fin spine and therefore after resorption may not be observed (and counted). This process is commonly observed but not constant or predictable and is likely to increase with age (Cort 1991) so, while making age estimates from fin spines, the chance of not observing the first (inner) increments increases with age.

Otoliths are immune from this resorption process so may be the most reliable structure for age estimates if it can't be determined whether inner growth increments in fin spines are being obscured. Assessing bias in otolith and fin spine age estimates is particularly useful to determine if the first one or two increments in fin spines are being counted. The lack of bias detected in our comparison of structures for ages 1-7 suggests that the first few increments are being counted in spines. However this analysis assumes that the first increment in otoliths is correctly assigned. There is uncertainty in determining the position of the first growth increment in otoliths but is important so that ages are not being consistently over- or underestimated. The comparison of daily increment counts (Kernandel et al., 2006) with the positions of annual increments in the current study, however, suggests that that the first two annual increments are being successfully identified in our sectioned otoliths (see Fig. 3.5 above).

Both structures have clear growth increments that provide reproducible counts, although a final age could be given to more fin spines than otoliths, suggesting better clarity in spines. But without direct validation of the timing of formation of growth zones it is impossible to determine which is most accurate. Langely (2006) reported that the longest time at liberty for a tagged albacore to date is 11 years. This fish is likely to have been between 12 and 15 years old as the fish was tagged as a juvenile aged 1-4 years (Langley and Hampton, 2005). These age estimates are consistent with the current study, as the oldest age estimated from fin spines and otoliths was 11 and 14 respectively.

Despite the differences in our age estimates between otolith and fin spines, the von Bertalanffy growth parameters obtained for both structures are very similar (Fig 3.8). Interestingly, a significant difference in growth between males and females was found in both

the otolith and spine data. Sexual differences in growth have also been found in other studies on albacore (Megalofonou, 2000; Santiago and Arrizabalaga, 2005).

Length-at-age obtained in our study is greater that that used in the current stock assessment (Langley and Hampton, 2005), and that obtained by Labelle at al. (1993) using direct age estimation of vertebrae and analysis of length-frequency data (Fig. 3.8). However, our length-at-age is similar to mean length-at-age estimated from data presented in Kerandel, et al. (2006; Table 1, n = 54) based on otolith readings. A maximum age of 9 years was obtained in that study. The estimates of t_o obtained in the current study are lower than expected and is due primarily to the absence of small fish (<75 cm) in our samples resulting in slightly higher estimated length-at-age for young fish (≤2-year age class).

It is recommended that cross-reading of otoliths be undertaken between CSIRO and SPC to determine if the otolith reading techniques are similar.

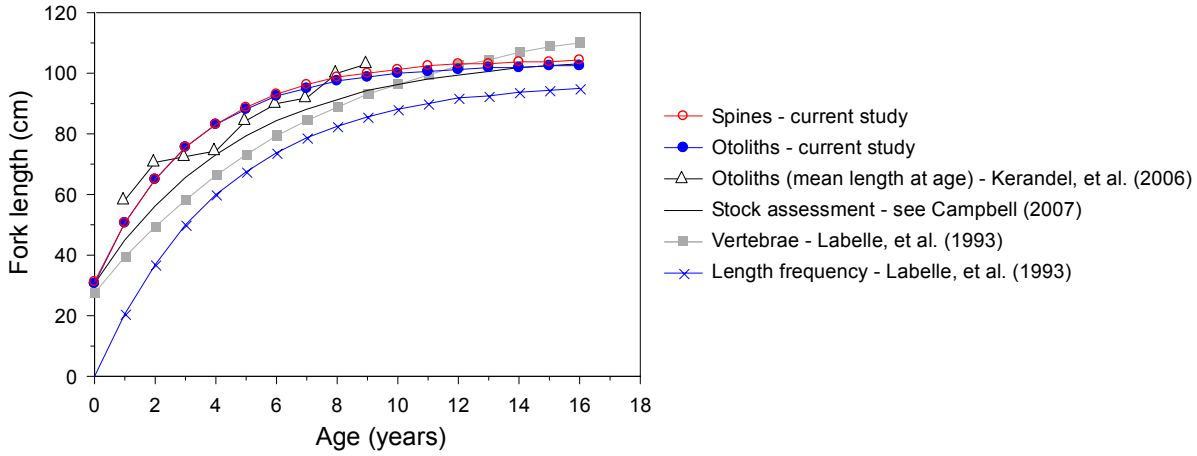

Fig 3.8. Growth curves obtained (von Bertalanffy or mean length at age) for South Pacific albacore.

3.5 Reproduction

Reproductive state of females

Ovary samples were collected from 62 fish measuring between 48 and 102 cm FL. The majority of females sampled off Queensland were mature (85.2%) and many were actively spawning (n=14; Table 3.7). This is the first record of actively spawning albacore from the Coral Sea, and they were all sampled in the 'albacore area' in the northern ETBF. The ovaries of all but one actively spawning female contained evidence of two spawning events; postovulatory follicles with either migratory nucleus (n=9) or hydrated (n=4) oocytes. Several mature but regressing (n=5) and regenerating (n= 26) females were also sampled, as well as 8 immature or developing females.

The majority of ovaries collected from eastern Tasmania (in March) were from fish between 48 and 74 cm FL, with gonads weighing less than ~10 g. These females were all classed as immature as the MAGO was unyolked and there was no histological evidence of atretic yolked oocytes (including delta atresia). One large (102 cm FL) female was also sampled off

Tasmania (in April). The most developed oocytes in the ovary were also unyolked, but delta atresia was present indicating that the fish had spawned previously and was in a regenerating state.

Although ovary weight was not obtained for all fish, there were clear differences in development based on the ovary weight data obtained (Fig. 3.9). Females less than 85 cm FL showed no or minor ovary development and it appears that they would not spawn in the near future. The majority of spawning capable or actively spawning females had a GI>1.7. However, two actively spawning females had GI values of 1.1 and 1.3; one also had 10-50% alpha atresia suggesting that it may be near the end of spawning, while the other had <10% alpha atresia. The ovaries of most actively spawning females were between 150- 330 g (Fig. 3.9) and were of similar or greater size to those sampled in the only other published data for the area (Isii and Inoue, 1956) where two ovaries weighing 289.1 g and 101.5 g were collected in January 1954 (17-20ºE, 152-153ºS). All immature, developing, regressing and regenerating females had a GI <1.7, apart from one regressing female with a GI of 1.75. The data suggests that while GI may be useful for examining spawning seasons and areas, it cannot be used to determine individual maturity levels or spawning activity.

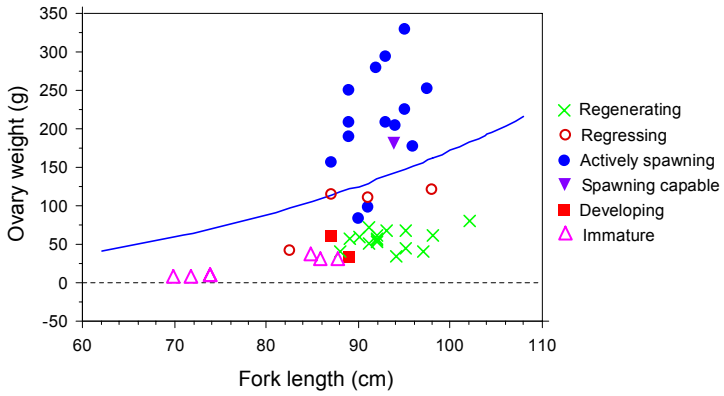

Figure 3.9. The relation between ovary weight and length by reproductive state for females sampled in the ETBF. The line represents a gonad index of 1.7 indicating maturity for females (from Ramond and Bailey, 1996). Note that gonad weight was not obtained for all females.

Table 3.7. Number of female albacore tuna sampled by reproductive state (December 2006 to July 2007)

	Class	*Maturity*	*MAGO*[1]	*POF*[2] *age*	*Alpha atresia*	*Beta atresia*	*Gamma & delta atresia*	*Queensland*	*Tasmania*
1	Immature	Immature	Unyolked	N	N	N	N	5	7
2	Developing	Immature	Early yolked	N	N	N	N	3	-
3	Spawning capable	Mature	Advanced yolked	None	Y (<10%)	Y	Y	1	-
4	Actively spawning	Mature	Advanced yolked	>12 hrs	10-50%	Y	Y	1	-
			MN or hydrated	<12 hrs	N	Y	Y	1	-
			MN or hydrated	>12 hrs	0 or <10%	Y/N	Y/N	12	-
5	Regressing	Mature	Unyolked	N	100%	Y/N	Y/N	2	-
			Early yolked	N	100%	Y/N	Y/N	2	-
			Advance yolked	N	>50%	Y	Y	1	-
6	Regenerating	Mature	Unyolked	N	N	N	Y	22	1
			Unyolked	N	N	Y	Y	4	
	Total							***54***	***8***

[1] MAGO = most advance group of oocytes in the ovary.
[2] POF = postovulatory follicle
Y/N = either present or absent

Reproductive state of males

Testis samples were collected from 97 fish measuring between 50 and 108 cm FL. All small males (<80 cm) sampled off Tasmania were histologically classed as immature. The one larger (89 cm FL) male sampled off Tasmania had spermatozoa in some areas of the testis suggesting that it may have spawned previously.

Only 18 testes from Queensland were examined histologically. Of these, 12 contained spermatozoa in either the lobules or the sperm duct suggesting they were in spawning condition, although evidence of recent or imminent spawning was observed in only 7 of 10 testes where the sperm duct was visible. Six males were classed as post-reproductive based on the presence of collapsed lobules and/or lack of spermatozoa. One testis appears to be immature (89 cm FL).

Ueyanagi (1957) suggested that albacore testes >150 g were probably mature based on based on the presence of milt in the sperm duct. Only two males sampled in the current project had testes weighing >150 g (153.3 g and 150.6 g; Fig. 3.10), suggesting that this criteria is not suitable for determining maturity of males caught off Queensland. In fact testes as small as 15-30g had evidence of recent spawning.

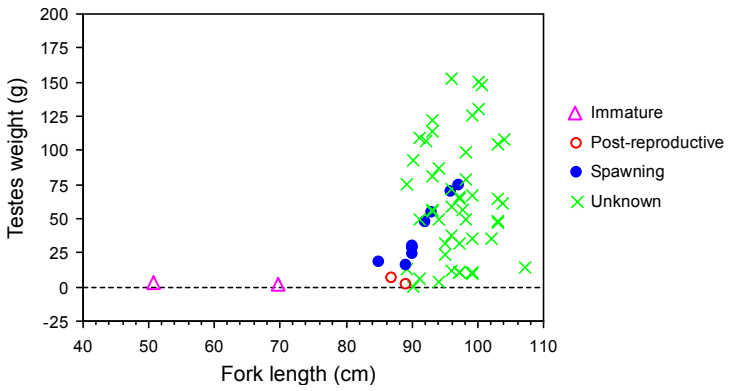

Figure 3.10. The relation between testes weight and length by reproductive state for males sampled in the ETBF. Note that testes weight and/or histological analysis was not obtained for all males

Spawning season

Although the sample size is small, histological analysis showed a cycle of ovary maturation between December 2006 and July 2007 from off Queensland (Fig. 3.11). In December 2006, two developing females were caught ~30ºS which is south of the likely spawning latitudes (10-25ºS). It is unknown if these females would have spawned in the coming season.

In January and February, 72% of females sampled were actively spawning. An additional 15-20% were in a regressing condition suggesting that they had finished spawning and that individuals may not spawn for the whole season. As only one spawning capable female was sampled during these peak spawning months, it appears that albacore may not rest between spawning episodes. By April, only one actively spawning female was sampled off Queensland, but as only 6 females were sampled in total, it is likely that spawning extends

until this month at least. In fact, this female had the largest ovary and GI sampled for the project (95 cm FL, 329.9 g ovary; GI=3.85). Several regressing females were also sampled in April, suggesting that the spawning season is nearing completion by this time. Ramon & Bailey (1996) report that female albacore from New Caledonian and Tongan waters spawn predominantly between November and February based on histological studies and the critical gonadosomatic index value of 1.7.

By June and July, over 90% of females sampled off Queensland were in a regenerating phase (identified by the presence of delta atresia). This suggests that some at least some females remain in the spawning area after spawning. However, the presence of a regenerating female off Tasmania in April confirms that some post-spawning females move away from spawning latitudes after spawning.

The preliminary histological data for males indicates a similar spawning season pattern to that of females. Actively spawning males were dominant in January and February. By April, some post-reproductive males were present and by June/July, almost all males analysed were post-reproductive.

Very few immature females are caught in the spawning area during the spawning season, and those that were sampled were caught in April, Jun and July rather than in the peak of the spawning season. It is possible that these females had recently migrated to the spawning latitudes during the cooler winter period to feed, or had moved in preparation for spawning the following season.

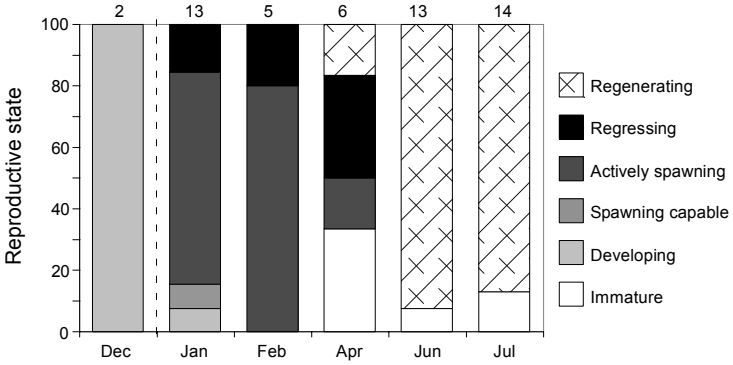

Figure 3.11. Reproductive state of female albacore sampled off Queensland by month based on histological analysis. Note sample sizes (shown across the top) are small for some months. December samples were collected ~30ºS and the remaining ~14-20ºS

Size at 50% maturity

As already mentioned, the criteria to distinguish mature from immature females is based on the presence of advanced yolked oocytes or atresia of advanced yolked oocytes (alpha to delta). Based on these criteria, 78.0% of females sampled in January to July were mature. Albacore with advanced yolked oocytes were sampled in January to April. Signs of delta atresia were present in ovaries in all months sampled, including June and July. The delta atretic bodies (see Fig 2.6) decreased in size between April and June, and again between

June and July. This is expected as the atretic bodies are slowly resorbed over time. It is unknown if delta atresia would be visible in ovaries collected in August or later.

Length at 50% maturity was estimated at 82.3 cm (Fig. 3.12). Using the estimated von Bertalanffy growth parameters for females based on otolith readings, a fish of this size would be 4 years old. Previous estimates of size at first (not 50%) maturity for Pacific albacore range from 82 cm (Ramond and Bailey, 1996) to around 90 cm (Ueyanagi, 1957; Otsu and Uchida, 1959). The current stock assessment uses age 5 years as 50% mature and age 6 years as 100% mature (Langley and Hampton, 2005).

It is important to note, however, that our calculation of length at 50% maturity is potentially biased because albacore caught in the ETBF off Queensland are predominantly large/mature females from the spawning latitudes. In these areas, immature albacore are underrepresented in the catch and thus the estimated L_{50} will be underestimated. Estimating size at 50% maturity is difficult for any species where the mature fish migrate to discrete areas to spawn, or where there is any bias towards mature fish in the sampling program. Sampling for maturity depends on both immature and mature individuals having been sampled in an unbiased way. Crone and McDaniels (2005) suggested a population-wide study was required to determine maturity schedules for females in the Pacific, and appropriate stratification is required to compensate for the spawning migrations. Our study has shown that there is a relatively long temporal window for sampling (January to July at least) which will allow for a clear distinction between mature and immature females for size at 50% maturity studies. Thus, sampling does not need to be restricted solely to the months or areas of spawning activity.

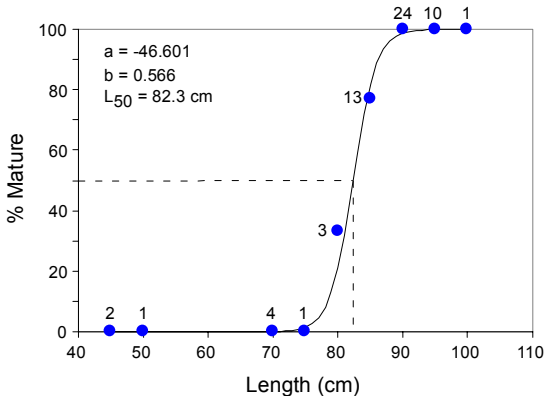

Figure 3.12. Percent of mature female albacore by 5 cm length class (n=59). Data restricted to January to July. Maximum likelihood fit of the logistic regression model is shown. Dotted lines show estimated length at 50% maturity. Note that the L_{50} estimate is potentially underestimated because sampling was biased towards mature individuals at spawning latitudes (see text above). Number of fish sampled is shown for each size class.

Spawning frequency

The ovaries of 14 (77.8%) of females sampled off Queensland in January and February (peak spawning months) had evidence of recent or imminent spawning activity. Another 3

were post-spawning and 1 was classed as immature. The fraction of mature females with postovulatory follicles was 0.76, giving a mean spawning interval of 1.3 days. Of females actively spawning, the fraction with postovulatory follicles was 0.93, giving a mean spawning interval of 1.1 days. The ovaries of 13 females gave evidence of two spawning events, that is, they contained maturing oocytes (either migratory nucleus or hydrated) and postovulatory follicles confirming that females can spawn daily.

Many tunas are reported to spawn almost daily such as skipjack (Hunter et al., 1986), bigeye (Nikaido et al., 1991; Schaefer et al., 2005), yellowfin (Schaefer, 1996) and southern bluefin tuna (Farley and Davis, 1998). Hsu and Chen (2005) reported that albacore in the western North Pacific spawn on average every 1.7-3.0 days in the peak spawning months of March and April. This study does not give the proportion of females sampled by reproductive class, or the spawning frequency for females actively spawning, making comparison difficult.

Batch fecundity

Oocytes in all development stages were present in the ovaries of spawning fish, confirming albacore have an asynchronous pattern of oocyte development and indeterminate annual fecundity. Counting the number of yolked oocytes present in the ovary prior to the spawning season will not give total annual fecundity. Asynchronous oocyte development has been found for many tunas, and is characteristic of species which spawn several times over a long spawning period.

Batch fecundity estimates for 7 females with hydrated oocytes ranged from 0.86 to 1.33 million oocytes (Table 3.8). The absence of new (0 hr) postovulatory follicles in these ovaries suggests that the stress of capture did not inducing the fish to release oocytes, and thus our batch fecundity estimates are not downward biased. Our estimates of batch fecundity, although variable, are similar to those by Ueyangai (1957) and Hsu and Chen (2005) for North Pacific albacore. Ueyangai (1957) sampled larger fish and subsequently had larger batch fecundity estimates (Fig 3.13). Fecundity counts by Isii and Inoue (1956), Otsu and Uchida (1959), and Wu and Kuo (1993) for Coral Sea, North Pacific and Indian Ocean albacore respectively, were not necessarily based on the number of migratory nucleus or hydrate stage oocytes present, and often included all vitellogenic eggs and would thus be higher than the true batch fecundity. The relationship between batch fecundity and fish size was not clear and may be due to inadequate sample size.

Relative batch fecundity ranged from 52.6 to 73.7 oocytes per gram of body weight, and the overall mean and standard deviation was 65.16 (±7.7) oocytes/gram of body weight. The mean relative fecundity is higher than has been found for bigeye tuna, *Thunnus obesus* (24 and 31 oocytes/gram of body weight) (Schaefer et al., 2005; Nikaido et al., 1991), but is similar to that found for southern bluefin tuna, *T. maccoyii* (57 oocytes/gram of body weight) (Farley and Davis, 1998), bluefin tuna, *T. thynnus* (59 oocytes/gram of body weight) (Medina et al., 2007), and yellowfin tuna, *T. albacares* (62-68 oocytes/gram of body weight) (Schaefer 1996; 1998; Sun et al., 2005).

Table 3.8. Batch and relative fecundity estimates for albacore tuna in the ETBF

Fork length (cm)	*Whole weight (kg)*	*Batch fecundity*	*Relative fecundity*
93	18.0	1,326,934	73.7
89	15.6	1,009,925	64.7
93	18.2	1,219,886	67.0
89	15.6	859,610	55.1
92	16.4	1,187,139	72.4
89	16.2	1,143,150	70.6
98	18.4	967,948	52.6
Mean		*1,102,084*	*65.2*

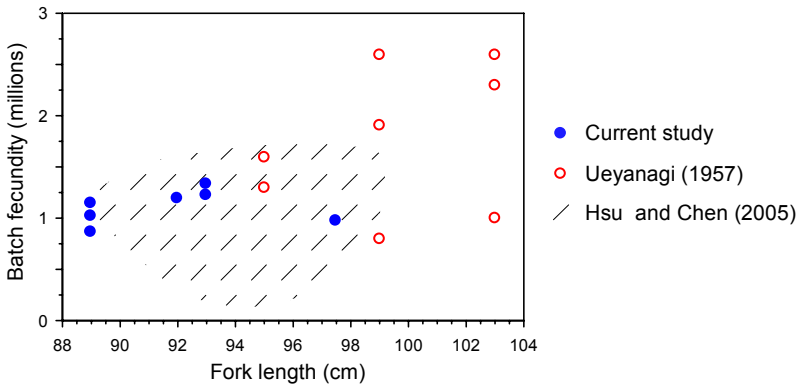

Fig. 3.13. Relationship between batch fecundity and fork length for albacore caught in the ETBF (current study) and the western north Pacific from Ueyanagi (1957; Table 3) and Hsu and Chen (2005; dashed area indicates the range of fecundity estimates for 21 fish from their Fig. 11).

4. Summary

This study has highlighted the need to undertake a large multi-year project to better define spawning behaviour including the timing and location of spawning aggregations, size/age at maturity, spawning frequency/duration, batch fecundity, and size-based reproductive outputs. For a larger multi-year project to be successful, sampling from the full size range of fish caught throughout the year is vital from boats that are targeting both albacore and other species on a regional scale. A regional project is currently in development to expand on this preliminary work.

5. Acknowledgments

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