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REGIONAL STUDY OF SOUTH PACIFIC ALBACORE POPULATION BIOLOGY: YEAR 3 – BIOLOGICAL SAMPLING AND ANALYSIS

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Jessica Farley[1], Ashley Williams[2], Campbell Davies[1], Simon Nicol[2]

[1] CSIRO Marine and Atmospheric Research, Wealth from Oceans Flagship, PO Box 1538, Hobart, TAS 7001, Australia.
[2] Oceanic Fisheries Programme, Secretariat of the Pacific Community, B.P. D5 - 98848, Noumea Cedex, New Caledonia.

Executive summary

This paper describes work undertaken in the third year of a regional study of South Pacific albacore population biology. The objectives were to complete biological sampling of albacore in the southwest Pacific region (otoliths and gonads), continue laboratory analysis of the material collected, and estimate preliminary biological parameters (age, growth and reproduction).

The biological sampling component of the project is complete with material being collected from 3,384 albacore caught across the southwest region from Australia to south of the Pitcairn Islands (i.e. from 130°E to 130°W). Very good industry cooperation was integral to the success of the sampling program. All material sampled has been received and archived for current and future use.

Preliminary length-weight relationships have been calculated for albacore sampled in Australia and New Zealand based on 1,756 measurements.

Validated (direct and indirect) otolith-based ageing protocols have been developed for albacore. Otoliths from 2,152 fish have subsequently been selected for annual age estimation based on sampling location, fork length and sex. All otoliths have been prepared (sectioned) and approximately half have been read. Daily ageing of small fish is also being undertaken to further validate the annual ageing protocols and to examine growth in the first year of life.

Histological sections of ovaries have been prepared for all females >70 cm fork length sampled (n=1,162). This size range encompasses immature and mature fish which is important for examining reproductive characteristics such as size/age-at-maturity, spawning time/area, and spawning fraction. All sections have been read and the reproductive status determined.

The priority for the next 5 months is to complete the laboratory work and analyses. Biological parameters will be delivered to stock assessment and harvest strategy scientists by the end of 2011.

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1. Introduction

In 2006, a regional age-based stock assessment for South Pacific albacore estimated the stock to be well above the level corresponding to the average maximum sustainable yield (MSY) and fishing mortality rates to be much lower than required to produce the MSY (Langley and Hampton, 2006). The stock assessment, however, used many biological parameters that were either uncertain or assumed (Hoyle, 2008). In 2006, a pilot project was undertaken in Australia's ETBF to provide preliminary descriptions of a number of these biological parameters for albacore (Farley and Clear, 2008), some of which were examined in the 2008 stock assessment and sensitivity analysis (Hoyle et al., 2008). The 2008 stock assessment was more pessimistic than the 2006 assessment (Anon, 2008) and it was recommended that substantially more biological samples are required to provide age-based estimates of population parameters to address the biological uncertainties in the current stock assessments for albacore in the South Pacific (Hoyle et al., 2008; Hoyle, 2008).

CSIRO subsequently proposed a 3-year project on albacore population biology in collaboration with SPC who had obtained EC funding for albacore biological research (SCIFISH project). The Western and Central Pacific Fisheries Commission Scientific Committee (WCPFC-SC) recognised that the project had "strong assessment implications with wide-spread benefits to a number of fisheries active in the WCPO". In 2008, it recommended and the Commission approved funding towards the first phase of the project to develop and implement a biological sampling program for the southwest Pacific. In 2009 and 2010, the WCPFC-SC again recommended and the Commission approved funding towards the second and third phases of the project to continue biological sampling, otolith reading and histological analysis of gonads. This paper describes the preliminary results of the project to-date.

2. Methods

2.1 Sample collection and processing

The biological sampling program for albacore across the southwest Pacific was completed in January 2011. In total, 3,384 albacore were sampled between 2009 and 2011 (current project) and 3,820 since January 2007 (Table 1). Material was obtained from albacore caught off Australia, New Zealand, New Caledonia, Fiji, Tonga, American Samoa, Cook Islands, French Polynesia, and south of the Pitcairn Islands. Very good industry cooperation was integral to the success of the sampling program - collecting material over such a wide geographic area (130°E to 130°W) will allow us to investigate regional variation in biological parameters such as age and growth. An additional two fish were sampled in New Zealand that had been previously injected with oxytetracycline (OTC) on a known date during conventional tagging operations (see age validation below).

The majority of fish sampled were measured to the nearest cm (fork length) apart from 93 fish sampled in Fiji that were measured to the nearest (lower) 5-cm. Whole weighed was measured to the nearest 0.1 kg for most sampled in Australia and New Zealand. The size distribution of fish sampled by region and sex is shown in Figure 1. Note that very few females were sampled in some regions - e.g. Fiji. Capture information including location and date were obtained for all fish while additional information such as life status and time of death were obtained for a subset.

The primary objective of the biological sampling program was to collect sagittal otoliths and gonads from each fish and to collect dorsal spines where possible. Table 2 shows the number of biological samples obtained by region. All biological material was sent to CSIRO Laboratories in Brisbane or Hobart where they were processed. Otoliths and spines were cleaned, dried and archived in CSIRO's 'Hardparts' collection. Gonads were weighed to the nearest 0.1 g (if whole), sex determined, and a subsample preserved in 10% buffered formalin for potential histological examination.

Table 1. Number of albacore sampled by month. Note that some fish may have only had one type of sample collected (otolith, gonad or dorsal spine). Excludes 2 recaptured fish injected with OTC for age validation.

Year-month		Aust[1]	NZ[2]	New Cal[3]	Fiji[3]	Tonga[3]	American Samoa[3]	Cook Islands[3]	French Polynesia[3]	South of Pitcairn Is[3]	Total
2007	Jan	36	18								64
	Feb	24	11								35
	Mar	26									26
	Apr	20									20
	May										0
	Jun	74									74
	Jul	36									36
2008	Jan		40								40
	Feb		40								40
	Mar		40								40
	Apr		40								40
	Aug	2									2
	Nov	29									29
	Dec										0
2009	Jan	33	34								67
	Feb	54	28								82
	Mar	87	10	48							145
	Apr	21		6							27
	May	36		19							55
	Jun	122		63							185
	Jul	112		12							124
	Aug	53		41							94
	Sep			34							34
	Oct	47		2			15		126		190
	Nov	30			48		4		27		109
	Dec	49			4				23		76
2010	Jan	82	40		45						167
	Feb	81	40					15			136
	Mar	72	40		111						223
	Apr	82	60								142
	May	78	46	42							166
	Jun	106				12	6		99		223
	July	65				121			97		283
	Aug	71									71
	Sep	40								56	96
	Oct	20					360	30		26	436
	Nov	20					44	83			147
	Dec	20					1	62			83
2011	Jan				23						23
Total		**1628**	**487**	**267**	**231**	**133**	**430**	**190**	**372**	**82**	**3820**

[1] Coordinated by CSIRO, [2] Coordinated by MFish, NIWA and SPC, [3] Coordinated by SPC.

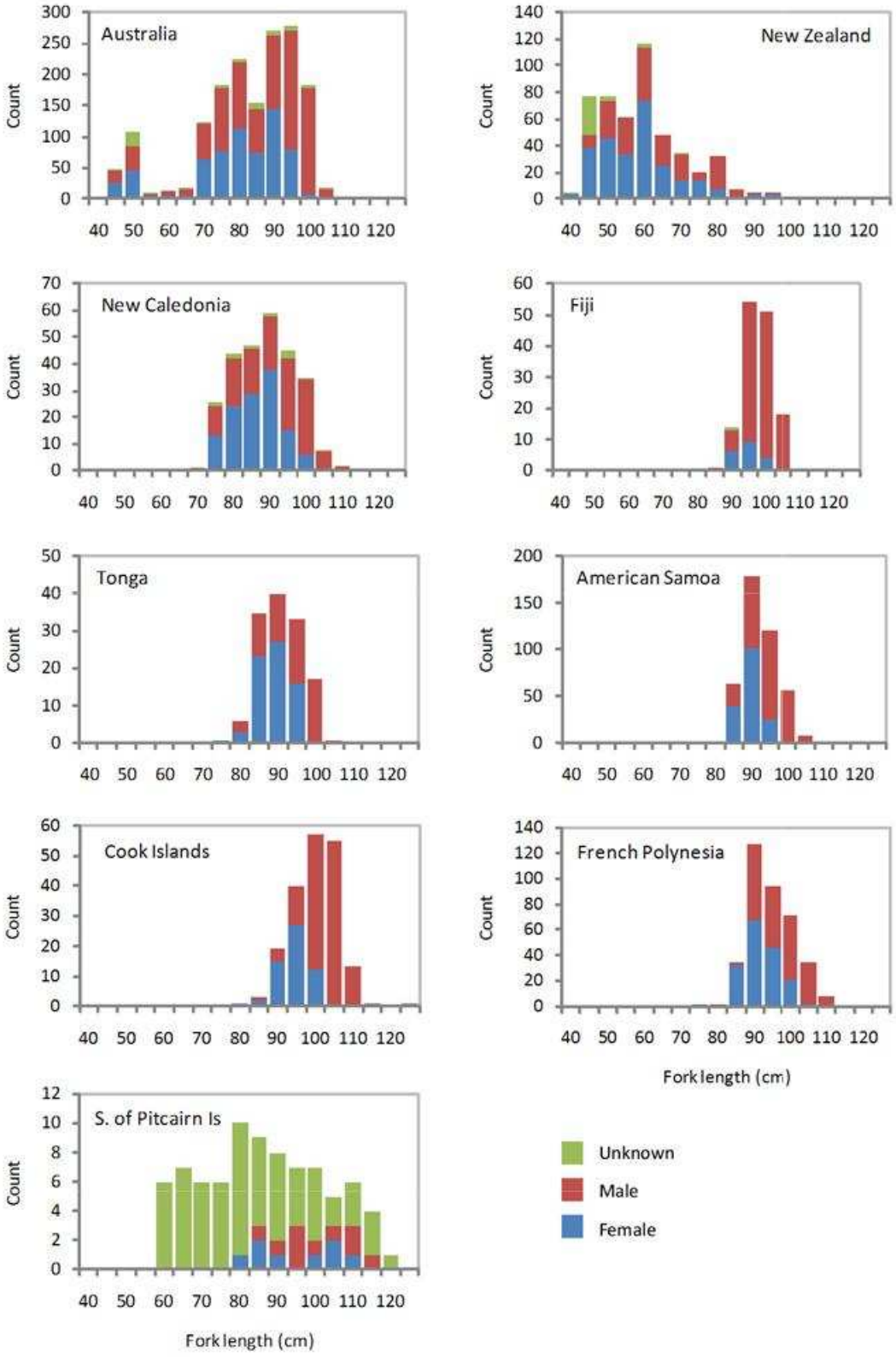

Figure 1. Length distribution (cm) of albacore sampled by region since 2007 (n=3820). Excludes 2 recaptured fish injected with OTC for age validation

Table 2. Number of biological samples collected by region since 2007. Only fish that had biological samples suitable for analysis and associated capture data are included. Excludes 2 recaptured fish injected with OTC for age validation

Region	Gonads	Otoliths	Spines	Gonad & otolith
Australia	1512	1478	1599	1402
New Zealand	448	473	372	436
New Caledonia	256	251	84	243
Fiji	224	210		203
Tonga	133	128	131	128
American Samoa	404	420	270	394
Cook Islands	186	168		164
French Polynesia	363	326		317
Sth of Pitcairn	9	74	61	9
Total	**3535**	**3528**	**2517**	**3296**

2.2 Length-weight relationship

Preliminary length-weight conversion factors were developed using data collected in Australian and New Zealand. The length-weight relationship is typically given as:

$W = a \times FL^b$ where W is whole body weight in kg, FL is fork length in cm, and a and b are parameters of the power function. Parameters were estimated using least-square linear regression of natural log transformed length and weight data.

2.3 Annual age estimation

2.3.1 Otoliths

Fish for age estimation were selected from those sampled since 2009. Fish were selected based on sampling location, fork length and sex with the aim of estimating age for the full size range caught in each fishery/region. Only fish with lengths measured by 1-cm intervals were included. For regions where low numbers of otoliths were collected, all otoliths were selected for ageing. For the other locations, otoliths were sub-sampled from the length distribution stratified by 1-cm length class. All otoliths were selected from length classes with small sample sizes, and a fixed number of otoliths were randomly selected from each of the remaining length classes. In addition, otoliths were selected for all remaining females ≥70 cm sampled in Australia to ensure that age-based reproductive parameters could be estimated (see reproduction section below). In total, 2152 fish were selected for annual age estimation (Table 3).

Left or right otoliths were selected randomly for each fish (with preference given to undamaged otoliths) and weighed to the nearest 0.1mg if complete. No significant difference in otolith weight was detected between left and right pairs, so either otolith could be used for age estimation (paired t-test, t = -1.308, p=0.193, n=152).

Most otoliths were prepared for reading by Fish Ageing Services (FAS) Pty Ltd. Otoliths were sectioned following the protocols developed for southern bluefin tuna (Anonymous, 2002). Each otolith was embedded in clear casting polyester resin and four serial transverse sections were cut (one section including the primordium) and polished to 0.40 mm before being mounted on glass slides. Some otoliths (n=118), however, were prepared by Tropical Fish Ageing and only one section was prepared for each otolith.

Opaque (dark) and translucent (light) bands are visible along the ventral 'long' arm of each otolith section under transmitted light which together are assumed to form one growth increment. The number of opaque zones are counted using the techniques developed for southern bluefin and bigeye tuna (Anon., 2002; Farley et al., 2006). A confidence score of 1 (poor) to 5 (excellent) was assigned to each reading. Increments at the terminal edge of the otolith were counted only if a translucent edge was visible after the increment.

Otoliths were read two or three 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 Average percent error (APE) and coefficients of variation (CVs) (Beamish and Fournier, 1981; Chang, 1982). To date, 1120 otoliths have been read.

2.3.2 Spines

To compare age estimates from different hardparts, dorsal fin spines were also selected from 279 fish sampled from across the region based on fish length where an otolith had already been selected for ageing. The spines were sent to FAS for sectioning following the protocols developed in Farley and Clear (2008). These spines will be read over the final 1-2 months of the project. Age bias and precision will be examined between reading from spines and otoliths.

Table 3. Number of albacore otoliths and spines selected for age estimation by region in 2009 and 2010.

Region	*Otolith annual age*	*Spine annual age*	*OTC injected*	*Otolith daily age*
Australia	757	100		53
New Zealand	192	43	2	20
New Caledonia	249	29		0
Fiji	125			0
Tonga	127	19		0
American Samoa	208	17		0
Cook Islands	168	17		0
French Polynesia	252			0
Sth of Pitcairn	74	54		0
Total	**2152**	**279**	**2**	**73**

2.4 Age validation

An important aspect of the ageing work is to validate the estimates obtained (accuracy). A combination of indirect and direct validation methods were used in the current study:

(1) examination of hardparts previously marked with chemicals, such as oxytetracycline (OTC), on a known date.
(2) daily ageing to confirm the location of the first annual increment and to examine the spread of birth dates.

(3) marginal increment ratio (MIR) analyses to determine if there is an annual cycle of increment formation.

2.4.1 Mark-recapture experiment (direct)

The best way to validate age estimates is to examine otoliths or spines previously marked with chemicals, such as OTC or strontium, on a known date. This allows quantification of the number of growth increments formed during the period the fish was at liberty. SPC is undertaking an age validation experiment for albacore using OTC to mark the hardparts of fish caught off New Zealand (Williams et al., 2009; 2010). Fortunately, two marked albacore have now been recaptured and the hardparts subsequently sampled. One of the otoliths has been sent to FAS for examination and the second will be sent as soon as possible.

2.4.2 Daily ageing (indirect)

Micro-increment analysis can be used to confirm the position of the first annual increment in otoliths assuming that the micro-increment counts represent daily age. The location of the first annual increment should occur on or before the location of the 365th daily increment. Preliminary analysis of 'sister' otoliths from 18 fish has been undertaken to examine the location of the first annual increment. The fish ranged in size from 43-56 cm FL. One transverse section was prepared from each otolith; one for daily ageing and one for annual ageing. For daily ageing, counts of micro-increments were made and measurements were taken from the first inflection point on the sectioned otolith to the 365th increment (age 1; Y1) and to the edge of the otolith. For annual ageing, counts of opaque growth zones were made, and measurements were taken from the first inflection point to the first opaque zone. All measurements were made along the external side of the ventral edge of the section. However, since the sister otoliths were likely to be sectioned at slightly different locations near the primordium, the distance from the first inflection point to the edge of the otolith were also slightly different. Thus the measurements from the otolith sectioned for annual ageing were scaled to the size of the sectioned otolith for micro-increment analysis so that direct comparisons could be made. The daily age estimates were also used to examine the spread of birth dates to compare with the known spawning season for albacore.

2.4.3 Time of increment formation (indirect)

Marginal increment ratio (MIR) analyses were undertaken to validate the annual periodicity of opaque zone formation in otoliths. Measurements for MIR were made on sectioned otoliths along the same axis that counts were made. The distance from the outer edge of the two most recently completed opaque zones to the edge of the otolith was measured. From these measurements, the MIR was calculated as the state of completion of the marginal increment as a proportion (%) of the previous increment (i.e. relative marginal increment). For otoliths with only one opaque zone, the absolute marginal increment was measured. These measurements were taken on all otoliths with clear increments at the terminal edge, although the measurements were probably most accurate for age classes 1-5 where the distance between opaque zones is still relatively large.

2.5 Reproduction

2.5.1 Female histological classification

Females for histological analysis were selected from those sampled since November 2008. Ovaries from all females ≥70 cm fork length were selected as this size range encompasses

immature and mature fish which is important for examining reproductive characteristics. This included females sampled in Fiji with lengths measured by 5-cm as these may be useful for describing some aspects of spawning dynamics including spawning locations, seasons and frequencies. Table 4 shows the number of ovaries analysed by region.

Table 4. Number of ovaries selected for histological analysis by region.

Region	***Number***
Australia	499
New Zealand	39
New Caledonia	125
Fiji	44
Tonga	70
American Samoa	161
Cook Islands	55
French Polynesia	166
Sth of Pitcairn	3
Total	**1162**

Tissue samples 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. Ovaries were classified using criteria similar to those developed for northern anchovy (Hunter and Macewicz, 1980, 1985a, b), skipjack tuna (Hunter et al., 1986), yellowfin tuna (Itano, 2000; Schaefer, 1998), bigeye tuna (Schaefer, 2005), and southern bluefin tuna (Farley and Davis, 1998) based on:

(1) the most advanced group of oocytes (MAGO) present – unyolked, early yolked, advance yolked, migratory nucleus, and hydrated.

(2) the presence and approximate age of postovulatory follicles (POF's) - absent, new, <12 hours, 12-24 hours.

(3) the extent of alpha atresia of advance yolked oocytes – absent, <10%, 10-50% 100%.

(4) the presence/absence of beta stage atresia

(5) the presence and level of macrophage aggregate material present – absent, minor, moderate, major.

(6) the presence/absence of other maturity markers (i.e. residual hydrated oocytes or encapsulated oocytes/follicular cysts.

The POFs were assigned ages based on criteria developed for skipjack, bigeye and yellowfin tunas (Hunter et al., 1986; Nikaido et al., 1991; Schaefer, 1996) all of which spawn in water temperatures above 24°C and resorb their postovulatory follicles within 24 hours of spawning. Albacore also spawn in these temperatures (Ueyanagi, 1969) and water temperature appears to be the dominant factor determining reabsorption rates (Fitzhugh and Hettler, 1995). Each female was classified into a maturity and reproductive class based on the criteria given in Table 6 (see Results).

2.5.2 Length/age at maturity

Length at 50% maturity for females were estimated by fitting logistic regressions to proportion of mature by 1 cm length class (or 5-cm for Fiji):

$P(\text{maturity} \mid FL) = (\exp(a+bFL)) / (1+\exp(a+bFL))$

where P is the estimated proportion of mature individuals at fork length FL, 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:

$L_{50} = -a/b$

Similarly, age at 50% (A_{50}) maturity was also estimated by fitting logistic regressions to proportion of mature by age class.

2.5.3 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 (1 day) to define the fraction of the population spawning.

2.5.4 Batch fecundity

Of the ovaries sampled, 192 contained migratory nucleus and 25 contained hydrated oocytes (and no new POFs) that could potentially be used to estimate batch fecundity. It is unlikely that ovaries with early stage migratory nucleus oocytes will be suitable for batch fecundity estimates because the stage has not clearly separated (by size) from advanced yolked oocytes. To date, subsamples have been taken from 34 ovaries with late stage migratory nucleus or hydrated oocytes for examination. A core subsample (periphery to the lumen) of between 0.05-0.08 g was taken from each ovary lobe (2 subsamples/fish). The subsamples were weighed to the nearest 0.01 mg and fixed in 10% buffered formalin.

Batch fecundity was estimated by the gravimetric method (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.

Additional gonads will be selected for batch fecundity depending on the analysis of the current samples.

3. Results

Given the amount of data obtained through the project, the full analysis will take some time to complete. Only preliminary data explorations and results are presented here and focus predominantly on Australia at this stage given the large sampling program and more advanced state of sample processing and analysis.

3.1 Length-weight relationship

Preliminary length-weight conversion factors for Australia/New Zealand are given in Table 5 and shown in Figure 2. The parameters in Table 2 were estimated using least-square linear regression of log-transformed data for all fish and by sex (Table 2). Although the L-W relationships are almost identical, analysis of covariance found a significant difference between sexes ($F = 65.88$; $P < 0.0001$) where females were very slightly heavier on average for their length compared to males. For example, females are 35g heavier on average at 50cm FL, and 560 g heavier at 100 cm. This difference may be due to the difference in the weight of gonads between the sexes and this will be investigated further. If the difference is biologically meaningless, conversion factors from data pooled across sexes can be used.

Table 5. Regression coefficients for preliminary length-weight (cm-kg) relationships for albacore sampled in Australia and New Zealand

Sex	a (intercept)	b (slope)	r^2	*n*
Female	1.06×10^{-5}	3.163	0.993	797
Male	1.11×10^{-5}	3.147	0.993	923
All	1.14×10^{-5}	3.143	0.993	1756

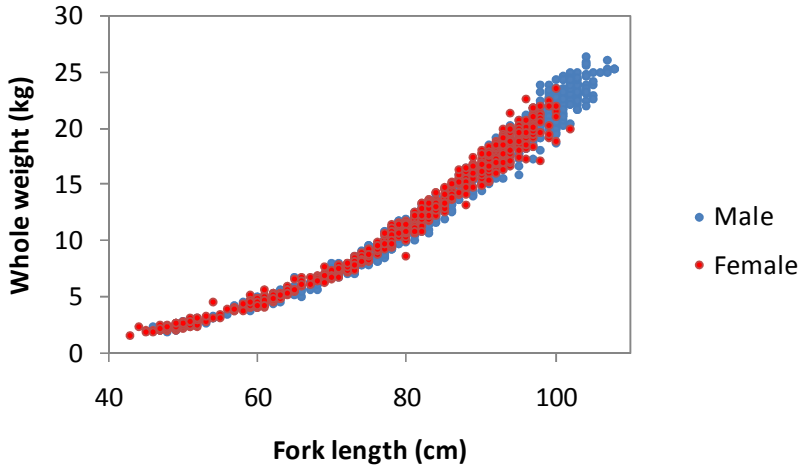

Figure 2. Plot of fork length (cm) to weight (kg) for albacore sampled in Australia and New Zealand since 2007 with known sex. n=1720.

3.2 Age estimation and validation

3.2.1 Otolith reading and precision

The clarity of annual increments in albacore otoliths varied between fish. The area closest to the primordium is generally difficult to read but the increments are relatively distinct and regularly spaced compared to other species like bigeye tuna. In some albacore otoliths, however, the terminal edge can be indistinct and difficult to interpret. Having multiple sections prepared for most otoliths has the advantage of at least one being clear enough to interpret and all sections can be assessed if necessary.

Of the 1120 otoliths examined so far, a final increment count has been assigned to 1025. The counts range from 1-14 for both males and females. The APE and CV of counts between blind readings was 4.71% and 6.66% respectively. When successive readings differed, 86.3% were only by ±1year indicating a reasonable level of precision.

All remaining sectioned otoliths will be read in the next 1-2 months.

3.2.2 Mark-recapture experiment (direct)

Examination of the otoliths from one of the OTC injected fish support the hypothesis of annual formation of opaque increments in albacore otoliths. Figure 3(A) shows two opaque increments with a third partially completed at the margin. Figure 3(B) shows that the OTC mark is clearly visible in the sectioned otolith and that this mark was deposited near the distal edge of the second opaque increment. This fish was injected and released on 28/2/2009 and recaptured on 16/2/2010. The amount of otolith growth in the 12 months subsequent to the OTC injection is consistent with expected otolith growth if opaque increments are deposited on an annual basis. If possible, counts of micro-increments after the OTC mark will also be undertaken.

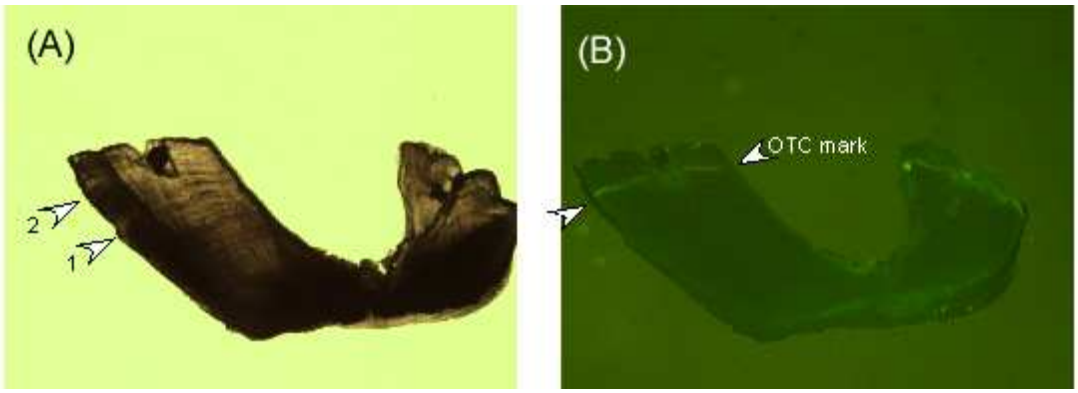

Figure 3. Transverse section of an albacore otolith injected with oxytetracycline (OTC) off New Zealand in 2009. (A) Location of the distal edges of the opaque increments counted under transmitted light with a partially completed opaque increment at the margin. (B) The OTC mark visible under ultraviolet light. Images taken by Fish Ageing Services Pty Ltd.

3.2.3 Location of the first annulus (indirect)

Micro-increment counts were obtained for 16 of the 18 otoliths examined and ranged from 185 to 552 (assumed) days. The age estimates were consistent with length-at-age estimates obtained in a previous study of South Pacific albacore (Kerandel et al., 2006). Back-calculated birth dates were also consistent with the known summer spawning season for albacore and ranged from early August to late April (mean of Jan 3). Note that in most cases, the sections were not prepared through the core of the otolith and it was likely that ~15-20 zones were missing from the counts for most otoliths. It is also not known when the first micro-increment is laid down in albacore otoliths (i.e. at hatching, a day after hatching, after first feeding, etc) but it is likely to be between 1-5 days after hatching as has been found in other tuna species (Jenkins and Davis, 1990; Itoh et al., 2000; Radtke 1983). The number of days between fertilization and hatching is also unknown but is likely to be only 1-1.5 days (Brothers et al., 1983).

Measurement to the 365th micro-increment (Y1) was obtained for 16 otoliths. Although obviously a measurement could not be obtained for the fish with only 185 increments, a measurement was obtained for an otolith that was not given a final increment count but a count was made up to the 365th increment. Comparisons of these measurements with the location of the first annual (opaque) increment in sister otoliths confirms that the first annual increment was successfully identified. The position of Y1 on the otolith occurred after the first opaque zone in 100% of sister otoliths. The average distance from the first inflection point to the first annual opaque zone was 660 µm, while the distance to the 356th increment (Y1) was 759 µm. This suggests that the 1st opaque zone formed on average just prior to the 1st birthday in these otoliths.

As already noted, otoliths from an additional 52 fish <56 cm FL are being analysed to continue this element of the project.

3.2.4 Time of increment formation (indirect)

The results of the preliminary MIR analyses (pooled data) indicate that an annual periodic signal exists (Figure 4). The mean MIR by month for fish in age classes 1-5 and all fish combined all show a sinusoidal cycle (vital for this type of validation) which indicates that one opaque zone forms per year, possibly between April and September. During these months, the MIR changes from being very high (opaque zone almost fully formed) to very low (new translucent zone forming). As more data are obtained, variability in the timing of increment formation will be examined by age class, location and year where possible. At this stage, however, we are confident that our data does contain an annual signal. Figure 5 shows examples of the monthly cycle of marginal increment in albacore otoliths.

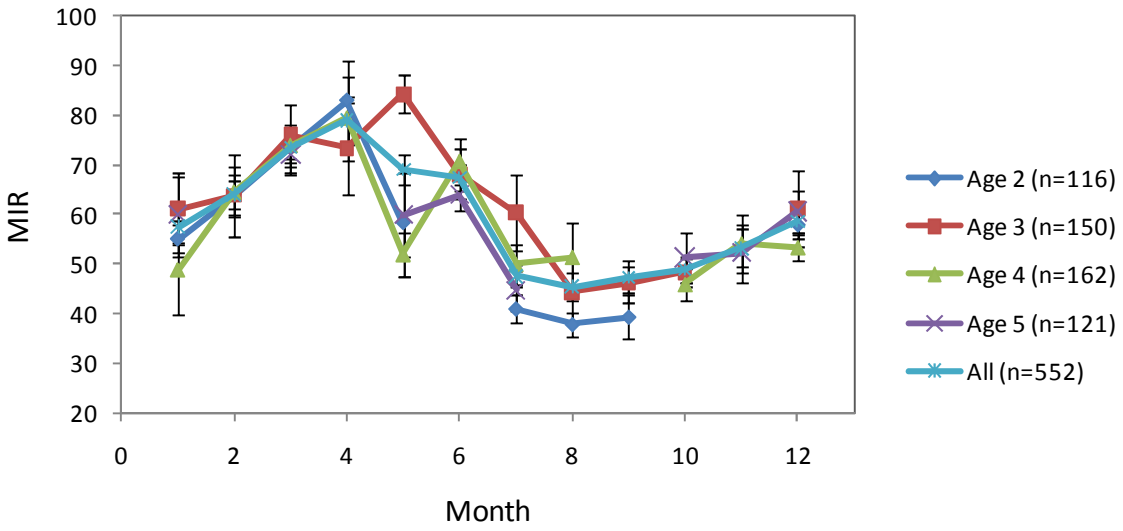

Figure 4. Mean of the monthly marginal increment ratio (MIR) for all albacore (+/-SE). Data are shown for age classes 1 to 5 and for all fish pooled where ≥ 5 measurements are obtained per month.

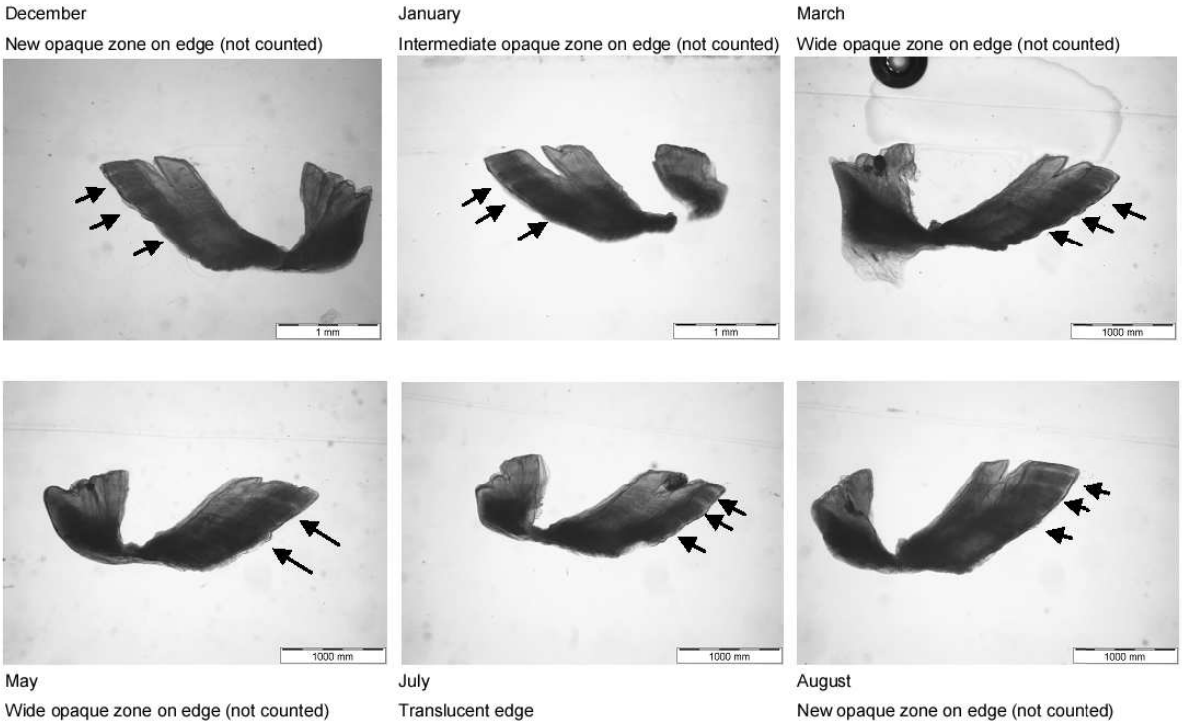

Figure 5. Examples of sectioned albacore otoliths showing different marginal increment widths. Arrows indicate opaque zones counted.

3.2.5 Decimal age

The MIR examined above is also useful for classifying the completion state (edge type) of the otolith margin, which is needed to determine the age class of each fish and to estimate decimal age. Rather than simply estimating an average date that the opaque zone is formed in albacore otoliths, we categorised each fish as either pre increment, post-increment or unknown based on the MIR (Figure 6). By doing this, we can infer whether an opaque zone had been deposited in the year of capture or not, which is especially important for fish caught between the months of May to September.

If, for example, an otolith sampled in those months had three opaque zones and a large MIR, then it was assumed that an opaque zone had not been deposited (or counted) that year. Alternatively, if the otolith had a small MIR, then it was assumed that an opaque zone had been deposited and counted. This must be taken into account when assigning a fish to an age class (age in whole years - number of birthdays). For otoliths with an intermediate MIR, it is impossible to determine whether the last opaque zone counted was deposited in the capture year or not.

As more data are obtained, variability in the timing of increment formation will be examined by age class, location and year. If significant variability exists, then this may lead to slightly different algorithms being developed to calculate decimal age (see below) by region or age class for example. At this stage of the project, the monthly cut-off MIR's for the three categories are preliminary (simply chosen by eye) but many be determined statistically as more data are obtained. However, it is important to note that these cut-off values increase each month over the May-Sep period.

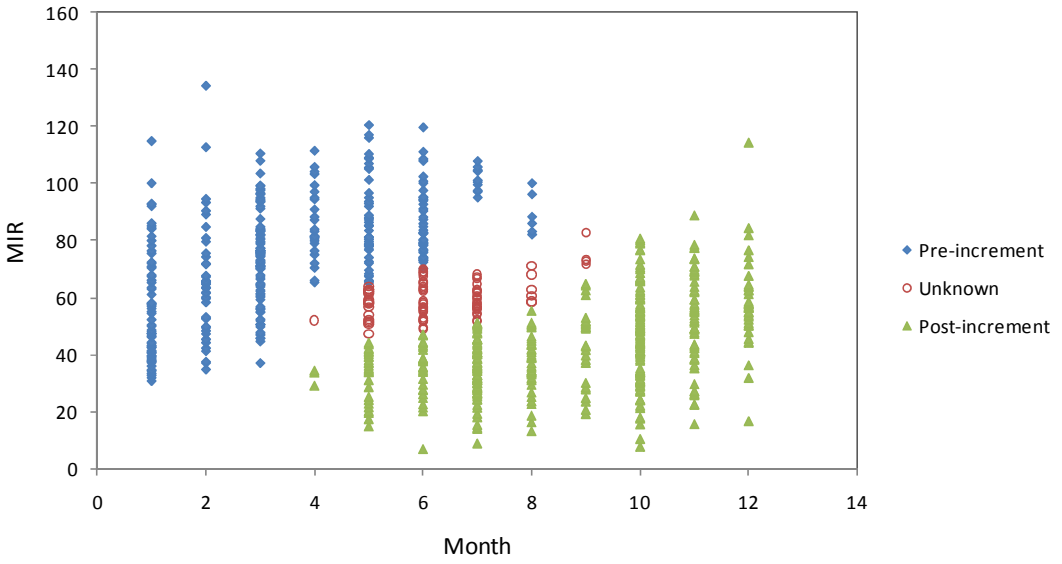

Figure 6. Marginal increment ratio values by month for albacore (all ages combined). The data are separated into three categories based on the MIR value: pre-increment, unknown, and post-increment (see text for explanation).

The decimal age for albacore was calculated using the opaque zone count, an average birth date of January 1 (middle of the spawning season), capture date, and the marginal increment ratio category (above). The current algorithm for all fish is:

- If a fish is caught between Jan and Mar OR if classified as pre-increment and caught Apr-Sep:

  Age = count + ((capture date - Jan 1)/365).

- If a fish is caught between Oct and Dec OR if classified as post-increment and caught Apr-Sep:

  Age = (count -1) + ((capture date - Jan 1)/365).

- Decimal age cannot be calculated for fish with an 'unknown' MIR category.

The average birthdate of Jan 1 may be updated after we examine the spread of birth dates from the daily ageing component of the project, and determine the length of the spawning season (see below). In fact, preliminary spawning data suggests that an earlier date may be more appropriate.

Figure 7 shows the currently estimated length at decimal age for albacore obtained so far, and clearly shows the differences between sexes. Interestingly, the maximum age is similar for both males and females, suggesting that the skew in sex ratio towards males in the larger length classes may in part be due to differences in growth (females don't grow as large) rather than sex-specific mortality or vulnerability to fishing. Each fish was also assigned an age class based on the number of birthdays.

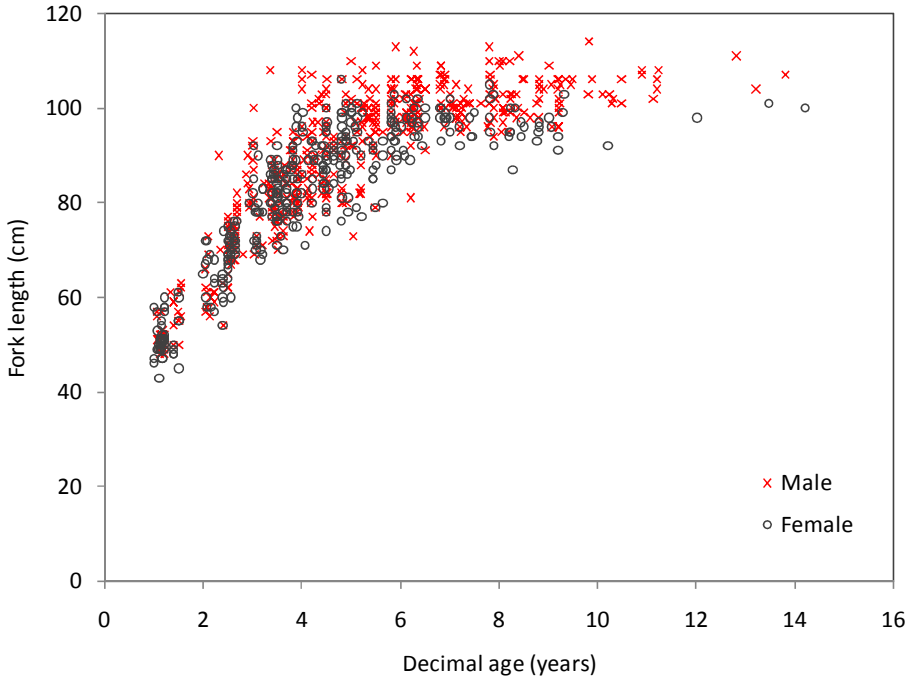

Figure 7. Preliminary length at (decimal) age by sex for albacore tuna (all regions combined) (n=885).

3.3 Reproduction

3.3.1 Sex ratio

As expected for albacore, males dominated the sampling in all length classes ≥90 cm fork length (Figure 8). The largest female sampled was 113 cm compared to 133 cm for males. The majority of females were ≤102 cm.

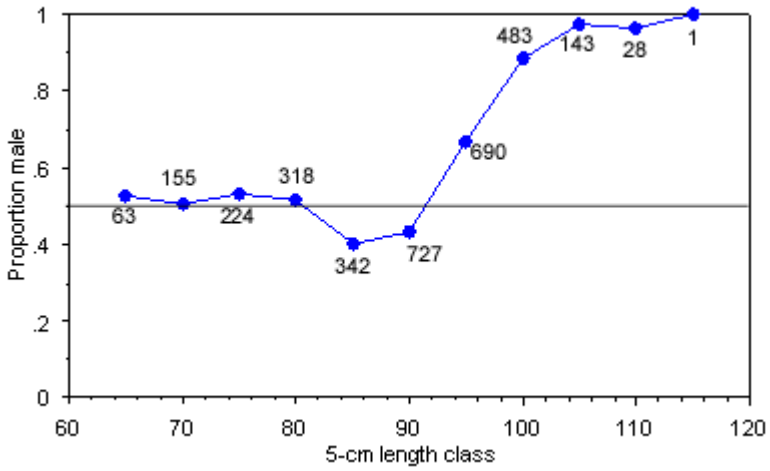

Figure 8. Proportion male by 5-cm length class for albacore >60 cm fork length (all regions combined). Sample size shown for each length class.

3.3.2 Female histological classification

Histological sections were prepared for 1,162 ovaries and a preliminary reproductive status determined for 1,148. The other 14 ovaries will need to be re-sectioned due to poor quality preparation. Where indicated, histological analyses of ovaries collected in 2007 in Australia (n=56; Farley and Clear, 2008) are included in the results below.

The criteria in Table 6 are precise enough to allow for mature but regenerating females to be distinguished from immature females after the spawning season. This is important for estimating size/age at maturity (see section 3.3.4 below). Regenerating females are identified by the presence of unyolked or early yolked oocytes (as the MAGO) and either

1. residual hydrated oocytes (Figure 9A), and/or
2. encapsulated oocytes/follicular cysts (Figure 9B), and/or
3. macrophage centres or aggregate (brown bodies) (Figure 9C, 9D),

as evidence of past spawning activity.

Residual hydrated oocytes are indicative of recent spawning while encapsulated oocytes/follicular cysts occur when the ovary does not release yolked oocytes which become encapsulated and slowly absorbed. These structures can remain in the ovary for months in some species (Tomkiewicz et al., 2003; Witthames et al., 2010; Domínguez-Petit et al., 2011; Armstrong and Whittames, In Press). Macrophage centres are immune cells in the ovary thought to be active in the breakdown of atretic oocytes and postovulatory follicles, and commonly aggregate within the ovarian connective tissue as dark yellow (brown bodies). We used these 'maturity markers' to indicate that the fish is sexually mature. The length of time that these 'maturity markers' remain in the ovaries is unknown but were detected in females in all months of the year.

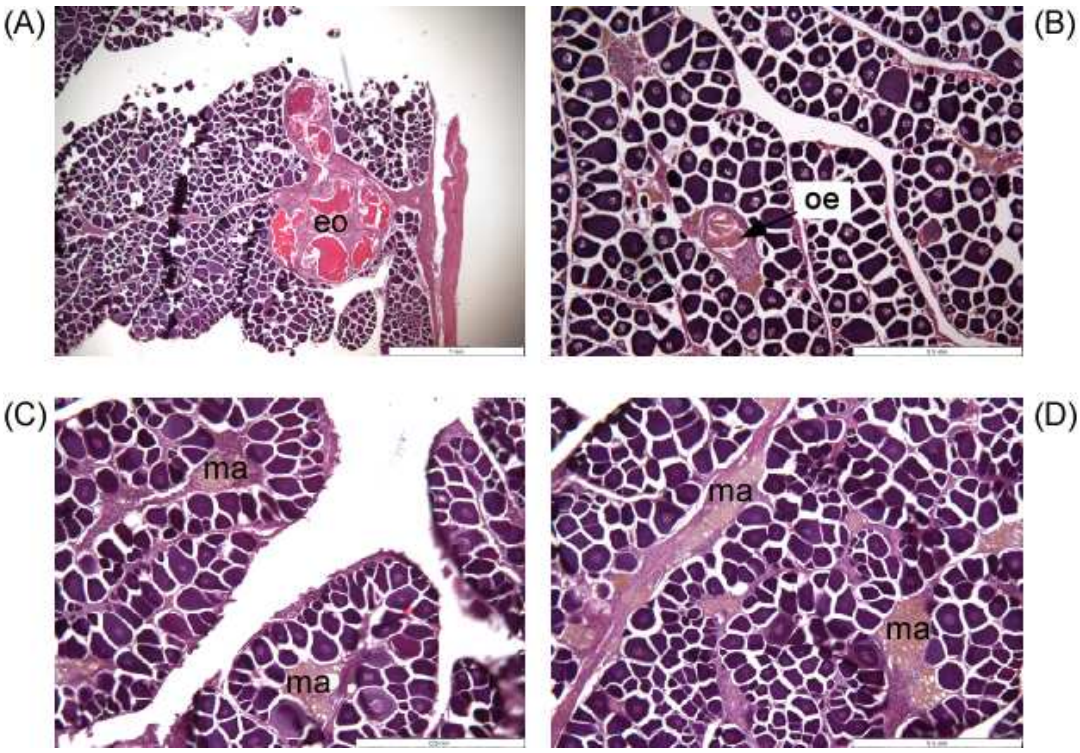

Figure 9. Maturity markers in regenerating ovaries. (A) Encapsulated hydrated oocytes, (B) encapsulated oocyte (eo), (C + D) macrophage aggregates (ma).

Table 6. Preliminary number of females ≥70 cm FL by reproductive class (all regions). Ovaries from the current project and Farley and Clear (2008; n=56) are included. Classification based on Itano (2000) and Brown-Peterson et al. (2010). MAGO = most advanced group of oocytes, POF=postovulatory follicle.

Class	Maturity status	Activity	Development class	MAGO and POF stage	Atresia of advanced yolked oocytes	Additional maturity markers[3]	Count
1	Immature	Inactive	Immature	Unyolked, no POFs	No alpha or beta atresia	None	359
2	Immature	Inactive	Developing	Early yolked, no POFs	No alpha or beta atresia	None	15
3	Mature	Active	Spawning capable	Advanced yolked, no POFs	<50% alpha atresia, beta atresia may be present	May be present	66
4	Mature	Active	Spawning	Migratory nucleus or hydrated and/or POF's	<50% alpha atresia, beta atresia may be present	May be present	354
5	Mature	Inactive	Regressing - potentially reproductive	Advanced yolked, no POFs	>50% alpha atresia, beta atresia present	May be present	9
6a	Mature	Inactive	Regressed (1)	Unyolked or early yolked, no POFs	100% alpha atresia, beta atresia present	May be present	28
6b	Mature	Inactive	Regressed (2)	Unyolked or early yolked, no POFs	No alpha atresia, beta atresia present	May be present	41
7	Mature	Inactive	Regenerating	Unyolked or early yolked, no POFs	No alpha or beta atresia. Gamma/delta atresia may be present	Present	332
Total							**1204**

[3] Maturity markers are residual hydrated oocytes, macrophage aggregates, or encapsulated yolked oocytes/follicular cysts

3.3.3 Spawning season and location

Active females (spawning capable or spawning) were caught in all regions apart from New Zealand (Table 7). The majority of spawning females, however, were sampled in Australia, American Samoa, the Cook Islands and French Polynesia. Spawning occurred predominantly between 12-20°S and the months of August to March.

The sampling program in Australia in terms of fish size, latitude and month allows for further investigation of spawning dynamics in albacore. Figure 10A shows that spawning occurs north of 20°S and that very few immature (or small) fish are caught at those latitudes. Active females were sampled from August to April, with peak spawning occurring in October to January. The data indicates that individuals do not spawn for the entire season as regressed (post-spawning) females were also sampled in August to April, but as expected their relative proportion increased towards the end of the season. Similarly, the proportion of regenerating females increased at the end of the spawning season and peaked in June and July well after the cessation of spawning.

Figure 10B and 10C shows that immature, regressing and regenerating females dominated the sampling at higher latitudes in Australia. The presence of regressing individuals between 21-30°S during the spawning months (October to February; Figure 10B) is consistent with individuals not spawning for the whole season and that some fish may move south (away from spawning latitudes) immediately after spawning. As expected, the proportion of regenerating females increased from March until September at higher latitudes and although the sample size is small, there is some indication that the abundance of regenerating females decreases gradually from September to December. This is consistent with mature females moving out of this region and north to spawn at the start of the spawning season. The majority of females sampled south of 30°S were immature although one regressed and several regenerating females were also sampled (Figure 10C).

Table 7. Preliminary number of females ≥70 cm FL by reproductive class and region. Ovaries from the current project and Farley and Clear (2008; n=56) are included.

	--Inactive--	**--------Active--------**		**----------Inactive----------**		
Region	Immature & Developing	Spawning capable	Spawning	Regressing - potentially reproductive	Regressed 1 & 2	Regenerating
Australia	275	20	75	5	34	144
NZ	31					8
New Cal	50	4	5		3	60
Fiji	4		15		2	23
Tonga	9	4	5	1	11	40
Am. Sam.		21	132			
Cook Is	1	6	44	2	2	
French P.	3	11	78	1	17	55
Sth of Pit.	1					2
Total	**374**	**66**	**354**	**9**	**69**	**332**

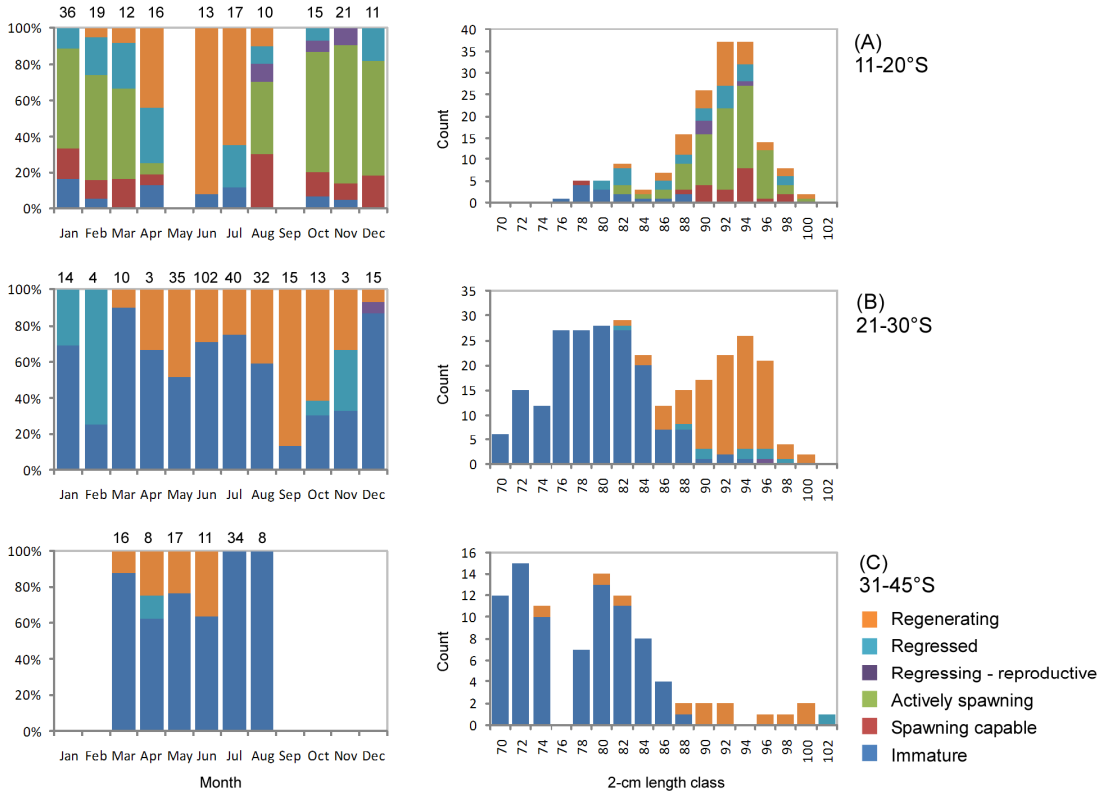

Figure 10. Frequency of development classes by month (left) and length (right) for females > 70 cm FL sampled in Australia. The data were separated by latitude (A) 11-20°S (n=170), (B) 21-30°S (n=285), and (C) 31-45°S (n=94).

3.3.4 Length/age at maturity

Figure 11A shows that gonads start to develop (increase in size) in females >85 cm fork length, although a few between 80 and 85 cm also show some development. The smallest mature female sampled was 74 cm fork length and the smallest spawning female was 78 cm. Figure 11B shows that although all females with a gonad index > 1.7 were mature, a large proportion of females below this level were also assessed as mature (i.e. regressing or regenerating) highlighting the importance of histological analysis of ovaries rather than using macroscopic criteria.

Albacore are somewhat unusual in that the size of fish caught generally increases with decreasing latitude. This is not surprising since adults spawn in tropical and sub-tropical waters, juveniles age 1 recruit to higher latitudes the following summer, and from there they gradually disperse north as they grow (Hampton and Fournier, 2000; Hoyle and Davies, 2009). As a consequence, the sampling program should encompass the full latitude range otherwise bias may be introduced towards mature or immature fish depending on the latitude sampled. For example, immature fish may be underrepresented at spawning latitudes and mature fish underrepresented at higher latitudes.

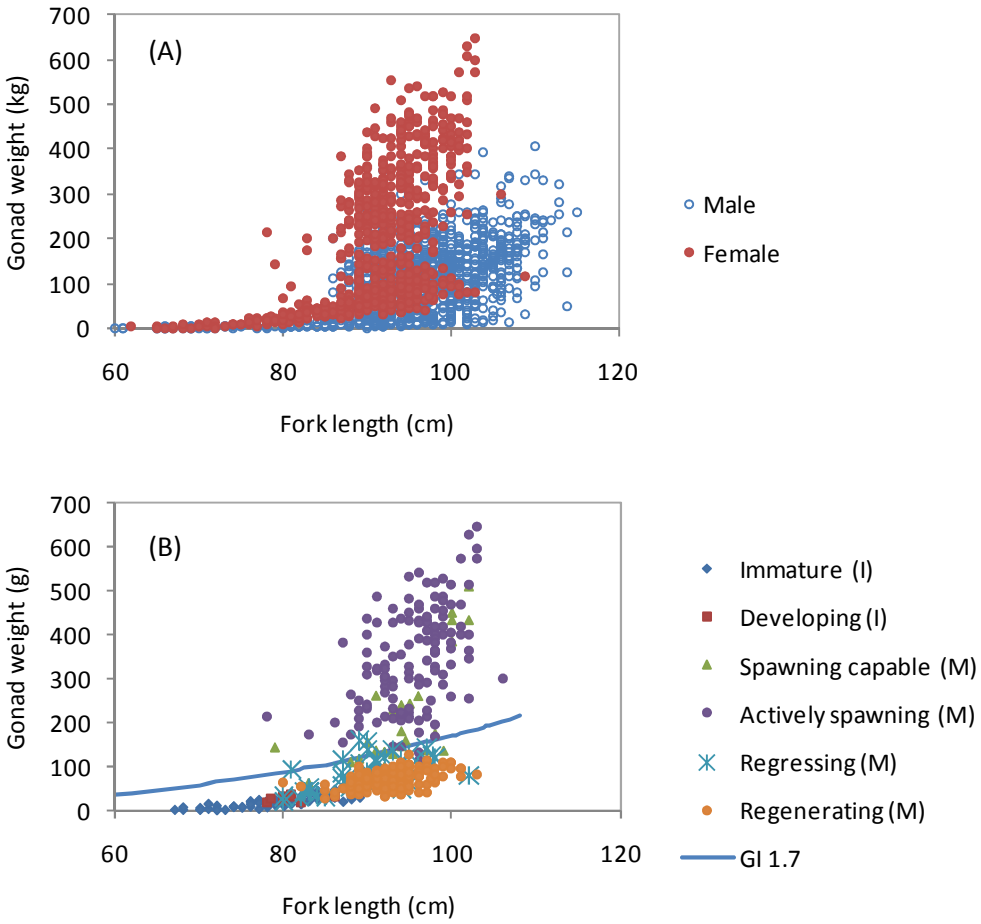

Figure 11. (A) Relation between gonad weight and length for albacore sampled during the project (n=940 males; n=757 females). (B) Relation between ovary weight and length by reproductive state for females M = mature and I = immature based on the reproductive state (n=511). The line represents a gonad index (GI) of 1.7 indicating maturity for females by Ramond and Bailey (1996).

Fortunately, sampling along Australia's east coast occurred from 14-43°S which covers most of albacore's longitudinal range of 5-45°S in the South Pacific. In our preliminary analysis, the Australian samples were divided into three latitudinal bands (11-20°S, 21-30°S and 31-45°S) and maturity ogives estimated for each (Table 8; Figure 12). The data were restricted to fish caught in specific months, at each latitude, to account for the times of year when immature and mature fish can be distinguished from each other. In the spawning latitudes north of 20°S, we used the months of August to April (spawning season). Note that if we included all fish at this latitude, the maturity ogive is almost identical suggesting that the criteria in Table 6 are specific enough to identify regenerating from immature females during the winter months. However, at higher latitudes only females sampled between December and July were included. Females sampled from August to November were not included because some regenerating females could be classed as immature given the length of time after the spawning season. Although preliminary, Figure 12 confirms that sampling location (latitude) does affect the estimated maturity schedule for albacore in Australia with the smallest L_{50} for fish sampled at 11-20°S (80.9 cm) and larger estimates to the south - 87.9 cm at 21-30°S and 89.3 cm at 31-45°S (Table 8).

Preliminary maturity ogives for several regions across the South Pacific show similar patterns to Australia although the small sample size in some areas means that the ogive may not be robustly estimated. The very small L_{50} for French Polynesia is due to only 3 immature females being sampled and again highlights the issues of sampling location affecting maturity calculations. Unfortunately no immature females were sampled in American Samoa and only one in the Cook Islands.

Additional work will be undertaken to model the maturity data (GLMs) with the different latitudes possibly weighted by a proxy for abundance of the length/age classes.

Table 8. Estimates of L_{50} for female albacore sampled in Australia by fitting logistic regressions to proportion of mature by 1 cm length class. $_1$ Samples from Fiji were by 5-cm length class, which will give a slightly smaller L_{50} than if 1-cm data were available. Note that sampling was not undertaken in all months in regions apart from Australia. Aust = Australia, FP = French Polynesia, NCAL = New Caledonia.

Region	Latitude	Months	N	L_{50}
Aust	All	All	553	86.3
Aust	11-20°S	Aug - Apr	140	80.9
Aust	21-30°S	Dec - Jul	222	87.9
Aust	31-45°S	Dec - Jul	86	89.3
FP	11-20°S	All	165	75.6
Fiji$_1$	11-20°S	Aug - Apr	44	82.5
Tonga	18-21°S	Dec - Jul	70	81.2
NCAL	18-25°S	Dec - Jul	79	86.1
NCAL	18-25°S	All	122	86.6

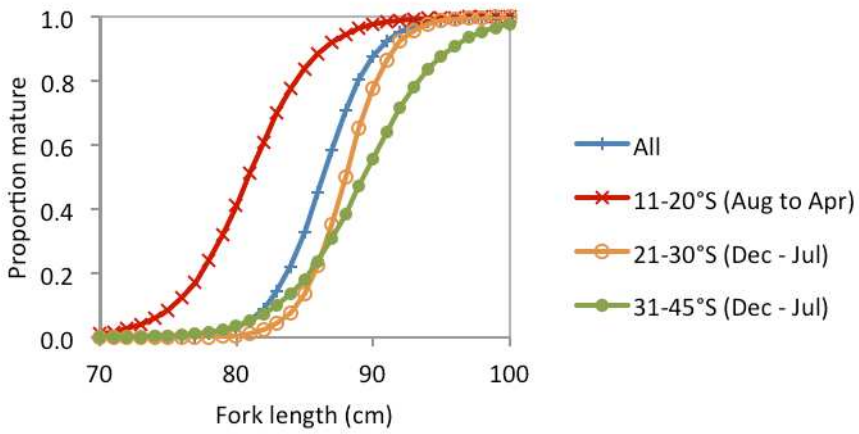

Figure 12. Maturity ogives for female albacore sampled in Australia (from Table 8).

3.3.5 Time of spawning and POF degeneration

Time of death was available for fish landed alive in American Samoa and the Cook Islands (longline caught) which allowed us to examine the daily cycle of oocytes maturation and POF degeneration in albacore. In these regions, the majority of live fish were landed between 4 pm and midnight, although a few were landed at other times. Of the females classed as spawning (n=58):

- advance yolked oocytes were apparent from 0551 until 1834 hrs (mean 1603 hrs)
- migratory nucleus oocytes were apparent from 1405 until 2325 hrs (mean 1933 hrs)
- hydrated oocytes were apparent from 1812 until 0022 hrs (mean 2056 hrs) (Figure 13A).

This suggests that spawning is synchronised in albacore and oocyte maturation is complete within 24 hours.

The ages assigned to POFs were also consistent with a 24 hour cycle in POF degeneration. Only one fish had POFs aged as 'new' and this fish was sampled just after midnight. The ovary also contained hydrated oocytes indicating that the fish was landed as it was about to spawn or that the fishing operation caused hydrated oocytes to be released. Although the sample size was low for the hours between 1am and 4 pm, POFs became progressively older during the day and all POFs assigned as >12 hours old were sampled after 2 pm (Figure 13B). POFs of different ages were not observed in the same ovary for any of the ovaries examined. The absence of 'new' POFs prior to midnight suggest that albacore spawn in the early hours of the morning.

Validation that POFs degenerate in 24 hours is important for calculating spawning frequency (below) using the postovulatory follicle method of Hunter and Macewicz (1985a).

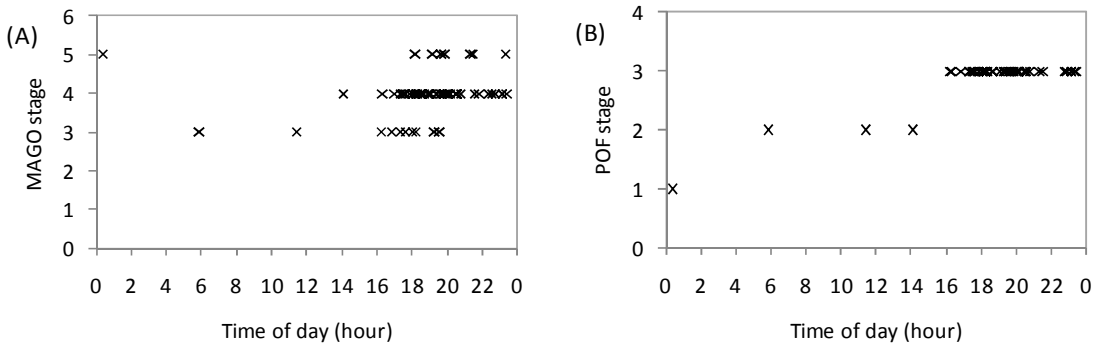

Figure 13. Relationship between time of death and (A) most advanced oocytes (MAGO) stage and (B) assigned postovulatory follicle (POF) age. Most advanced oocytes stages are: 3=Advanced yolked, 4=migratory nucleus, 5=hydrated. Postovulatory follicle stages are: 1 =new, 2=<12hours, 3=>12 hours.

3.3.6 Spawning frequency

Preliminary estimates of spawning frequency were calculated for females sampled in Australia during the spawning season (August and April) and the latitudes of 11-20°S. The fraction of all mature females sampled (n = 128) with postovulatory follicles < 24 hours old was 0.54, and a mean spawning interval of 1.86 days. If only females classified as active

(spawning capable or spawning; n = 95) are included, the fraction spawning per day is 0.74, giving a mean spawning interval of 1.35 days.

Initial investigations suggest that the frequency of spawning is highest in October to January (Table 9). The spawning frequency also appears to increase with increasing fish length (Table 10) suggesting that either small fish spawn less often during the spawning season or have a shorter season. A short spawning season is supported to some extent as only 22.2% of females in the 80 cm length class were reproductively active and the remaining 77.8% were inactive (i.e. post-spawning).This compares to the larger length classes where ~80% of fish were actively spawning. Further analysis will be undertaken to determine if bias is being introduced by other factors such as sampling month.

Table 9. Spawning frequency of mature females by month in Australia. Only fish sampled in the spawning latitudes between 11-20°S are included.

Month	N	N with POFs	Spawning fraction	Spawning frequency (days)
Aug	10	4	0.40	2.50
Sep	0	-	-	-
Oct	14	9	0.64	1.56
Nov	20	14	0.70	1.43
Dec	11	7	0.64	1.57
Jan	29	19	0.66	1.52
Feb	18	9	0.50	2.00
Mar	12	6	0.50	2.00
Apr	14	1	0.07	14.00
All	**128**	**69**	**0.54**	**1.86**

Table 10: Reproductive activity and spawning frequency of mature females by 5-cm length class in Australia. Only fish sampled in the spawning latitudes of 11-20°S and the spawning months of Aug-Apr are included. Active includes spawning capable and spawning females. Inactive includes regressing and regenerating females.

Length class (cm)	N	Active (%)	Inactive (%)	Number with POFs	Spawning fraction	Spawning frequency (days)
75	1	100.0	0	0	-	-
80	9	22.2	77.8	2	0.22	4.50
85	17	58.8	41.2	9	0.53	1.89
90	67	80.6	19.4	36	0.54	1.86
95	32	81.3	18.7	21	0.66	1.53
100	2	50.0	50.0	1	-	-
All	**128**	**73.4**	**26.6**	**69**	**0.54**	**1.86**

3.3.7 Batch fecundity.

Batch fecundity has been estimated for only 7 females so far. The number of hydrated oocytes ranged from 0.98 to 1.74 million oocytes.

4. Future work

Only preliminary data exploration and results are presented here. The priority for the next few months is to complete the laboratory work and undertake the final analyses which will also include information on regional and sex-specific differentiation in growth. Biological parameters will be delivered to stock assessment and harvest strategy scientists by the end of 2011.

5. Acknowledgements

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