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**Regional Study of South Pacific Albacore population biology:
Year 2 – biological sampling and analysis**

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Regional study of South Pacific albacore population biology: Year 2 - biological sampling and initial analysis

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Executive summary

This paper describes the results of the second year of a regional study of South Pacific albacore population biology. The main objective of this year of the study is to continue biological sampling of albacore in the southwest Pacific region, and to initiate otolith reading and histological analysis of gonads to ensure that unbiased estimates of biological parameters (age, growth and reproduction) can be obtained for albacore. These parameters are required inputs for regional stock assessment models and Australia's Eastern Tuna and Billfish Fishery (ETBF) harvest strategy.

Biological samples have been collected from over 2,500 albacore caught in the southwest Pacific since early 2007. Over the past 12 months, CSIRO continued to sample albacore caught in Australia's Eastern Tuna and Billfish Fishery (ETBF) off Queensland and New South Wales, with supplementary sampling from the recreational fisheries in Victoria and Tasmania. SPC continued to collect biological samples from albacore caught in the wider South Pacific through their SCIFISH project. Fish were sampled in New Caledonia, New Zealand, Fiji, American Samoa, French Polynesia, Tonga and the Cook Islands. In addition, New Zealand MFish and NIWA sampled albacore caught in New Zealand's domestic troll fishery over the 2010 summer fishing season. The majority of the biological material that has been sent to CSIRO has been processed in the laboratory (gonads), archived into CSIRO's 'hardparts' collection (otoliths and dorsal spines) or frozen (muscle tissue). Some material remains in the sampling ports waiting for sufficient material before freighting to Hobart.

Otoliths from fish caught in Australia, New Caledonia and New Zealand were sectioned for annual age (n=314) and daily age (n=18) estimation by laboratories in Australia. Age was estimated by 1-3 readers and an inter-laboratory comparison was undertaken to assess the level of precision of increment counts between readers/laboratories. An additional 219 otoliths and 100 dorsal spines were selected for ageing.

Histological sections prepared from 197 ovaries were read and staged. An additional 220 ovary samples have been sent for histological preparation. All ovaries selected for histological analysis so far were from fish >70 cm FL sampled in Australia, New Caledonia, New Zealand, Fiji, and French Polynesia.

Updated length-weight parameters were estimated for albacore caught in Australia and New Zealand. A significant difference was detected in the length-weight relationship between sexes where females were heavier on average for their length compared to males.

Table of contents

1.	Introduction	1
2.	Biological sampling program	1
2.1	Australian ETBF and recreational fishery	1
2.2	New Zealand seasonal troll and longline fisheries	2
2.3	Pacific Island Countries and Territories (PICTs)	2
2.4	Laboratory processing	3
3.	Direct ageing	4
3.1	Inter-laboratory comparisons (annual ageing)	4
3.2	Otolith reading (annual ageing)	6
3.3	Whole and sectioned otolith comparisons (annual ageing)	7
3.4	Spine reading (annual ageing)	8
3.5	Inter-laboratory comparisons (daily ageing)	8
3.6	Age validation	9
5.	Histological analysis of ovaries	10
6.	Length-weight relationship	11
7.	Future sampling	12
7.	Acknowledgements	12
8.	References	12

1. Introduction

Albacore tuna are found in waters between approximately 10-50°N and 5-45°S globally. Separate northern and southern stocks are assumed to exist in the Pacific Ocean based on their spatial distribution and different spawning times/locations. In the South Pacific, albacore have been targeted by longline fleets since the early 1950s, and recent catches have increased from 20,000-30,000 t per annum in the mid-1980s to 60,000-70,000 t per annum in the mid-2000s (Hoyle et al., 2008). The increase in catch has been largely due to an increase in the catch by small longline fisheries in Pacific Island Countries and Territories (PICTs). The catch of albacore in Australia's Eastern tuna and Billfish Fishery (ETBF) has also grown substantially from a few hundred tonnes prior to 2004 to 2,591 tonnes in 2006 and 1,916 tonnes in 2007. The species now represents a main target species for the ETBF fleet. These increased catches have raised concerns about whether these catch levels can be maintained long-term, the potential risk to the stock(s) and the potential reduction in the economic return from the fisheries.

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 the recent management strategy evaluation for albacore in the ETBF also suggested that the population is at low levels (Preece et al., 2009). It is 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 (Farley and Clear, 2008; 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 (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, the WCPFC-SC again recommended and the Commission approved funding towards the second phase of the project to continue biological sampling and initiate otolith reading and histological analysis of gonads. This paper describes the results of this second phase of the study.

2. Biological sampling program

2.1 Australian ETBF and recreational fishery

CSIRO continued to collect biological samples from albacore caught on the east coast of Australia. Fish caught in the ETBF were sampled at processors in Mooloolaba (Qld) and Coffs Harbour (NSW), while fish caught by recreational fishers were sampled in Portland

(Vic) and in several ports along the east coast of Tasmania. A total of 762 fish have been sampled since July 2009, and 1,382 since the start of sampling (Table 1). For each fish sampled, fork length (FL) was measured to the nearest centimetre and whole weight to the nearest 0.1 kg for most. Sagittal otoliths, the first dorsal spine and a muscle tissue sample were removed from each fish and frozen. Gonads were removed from the majority of fish and were either frozen whole, preserved whole in 10% buffered formalin, or a subsample removed and preserved in formalin before the remaining gonad was frozen. All material was sent to CSIRO. For each ETBF landing sampled, the vessel name and date were recorded so that the fishing location and additional data can be obtained from AFMA logbooks. Fish sampled in Australia ranged in size from 46-116 cm FL.

2.2 New Zealand seasonal troll and longline fisheries

The New Zealand Ministry of Fisheries (MFish) and the National Institute of Water and Atmospheric Research (NIWI) coordinated the collection of biological samples from albacore caught in the domestic troll fishery via their observer program (port sampling). Forty fish per month were sampled in January through April (n=160; Table 1). Fork length was measured to the nearest cm, and gonads, otoliths, the first dorsal spine and a muscle tissue sample were removed and frozen. Juvenile albacore again dominated the sampling in 2010 with the majority of fish ranging in size from 45-65 cm FL, although fish up to 76 cm FL were sampled.

During the second phase of their albacore tagging program, the Secretariat of the Pacific Community (SPC) collected biological samples from 66 albacore caught in the domestic longline fishery in April-May 2010 (Table 1). For each fish sampled, FL was measured to the nearest centimetre and otoliths removed and stored dry. The first dorsal spine, gonads, and a sample of muscle and liver tissue were removed and frozen. A sample of blood plasma was also taken from each fish and frozen for later analysis of sex hormones for correlation with maturity estimates. A wider range of sizes was sampled with longlines compared to trolling, with albacore sampled ranging in size from 60-100 cm FL.

2.3 Pacific Island Countries and Territories (PICTs)

The SPC continued to coordinate the collection of biological samples across the Pacific through their SCIFISH project. At least 670 fish have been sampled from New Caledonia, Fiji, American Samoa, French Polynesia, Tonga and the Cook Islands since Jan 2009, and over 800 since the start of sampling (Table 1). Fork length was measured to the nearest cm for most fish apart from 73 fish sampled in Fiji which were measured to the nearest 5 cm. Gonads and otoliths were removed from all fish. Dorsal spines were also removed from a proportion of fish sampled in New Caledonia and all fish from Tonga. All fish sampled in Tonga also had muscle, liver tissue and stomachs sampled, while blood plasma was obtained for 36 fish (see SPC's albacore tagging program report; WCPFC-SC6). All samples were frozen. Albacore sampled in the PICTs ranged in size from 74-114 cm FL.

A number of logistic issues have limited the number of samples collected from PICTs. The difficulties in adequately training a large number of observers in biological sampling methods and the prioritisation of observers for the purse seine fleet during the spatial closures restricted the number of observers actively collecting samples. Difficulties in ensuring proper handling and storage of samples during shipping have resulted in numerous samples being

destroyed. The 2009 tsunami prevented the collection of a number of samples from the Taiwanese fleet who were not able to offload in American Samoa.

Table 1. Number of albacore sampled by country or PICT since Nov 2006. Aust. = Australia, NZ = New Zealand, New Cal = New Caledonia.

Year	Month	Aust ¹	NZ ²	New Cal ³	Fiji ³	American Samoa ³	French Polynesia ³	Cook Islands ³	Tonga ³
2006	Nov	1							
2006	Dec	3							
2007	Jan	36							
2007	Feb	24							
2007	Mar	26							
2007	Apr	20							
2007	May								
2007	Jun	89							
2007	Jul	37							
<hr/>									
2008	Jan		40						
2008	Feb		40						
2008	Mar		40						
2008	Apr		40						
<hr/>									
2008	Aug	2							
2008	Nov	29							
2008	Dec								
2009	Jan	33	34						
2009	Feb	54	28						
2009	Mar	87	10	47					
2009	Apr	21		6					
2009	May	36		19					
2009	Jun	122		63					
2009	Jul	112		11					
2009	Aug	53		41					
2009	Sep			34					
2009	Oct	47		2		16	126		
2009	Nov	30			48	4	27		
2009	Dec	49			4		23		
2010	Jan	82	40		45	3		12	
2010	Feb	81	40						
2010	Mar	72	40	15	111				
2010	Apr	82	60						
2010	May	78	46	40					
2010	Jun	76							12
2010	July								99

¹ Coordinated by CSIRO (Australia)

² Coordinated by MFish/NIWA (New Zealand) and SPC (New Caledonia)

³ Coordinated by SPC

2.4 Laboratory processing

The majority of biological samples collected have been received by CSIRO Marine Laboratories in Hobart, Tasmania. Some material remains at the sampling ports waiting for sufficient material to be collected before shipment to Australia. In the laboratory, otoliths and fin spines are cleaned, dried and archived in CSIRO's 'Hardparts' collection. Gonads are weighed to the nearest 0.1 g (if whole), sex determined, and a subsample preserved in 10% buffered formalin for later histological examination (if not already done in port). Muscle

tissue and blood plasma samples are archived frozen. The size distribution of albacore sampled since November 2006 is shown in Figure 1.

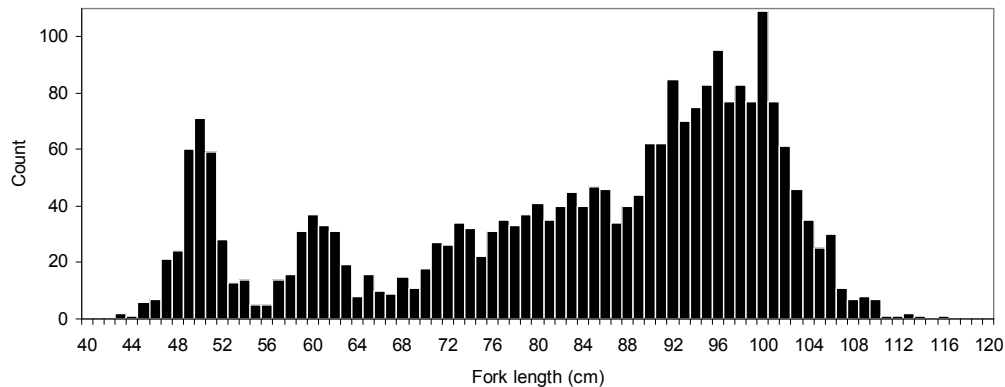


Figure 1. Size distribution of albacore sampled in Australia, New Zealand, New Caledonia, Fiji, French Polynesia, American Samoa, Tonga and the Cook Islands since November 2006 by 1-cm length class.

3. Direct ageing

3.1 Inter-laboratory comparisons (annual ageing)

Farley and Clear (2008) showed that albacore otoliths have clear growth increments (opaque zones) that are assumed to form annually, and found a good level of precision (consistency) between increment counts by the primary reader. It is important, however, in any ageing study to assess the level of precision of increment counts between readers and especially across laboratories.

In 2009, SPC sent 118 albacore otoliths sampled from New Caledonia ($n=48$) and New Zealand ($n=70$) to a laboratory in Queensland for sectioning and age estimation. One transverse section was prepared from each otolith, and three increment counts and a final age were provided by the reader (R1). R1 has considerable experience in otolith and ageing research, especially for tropical reef species, but not tunas.

The otoliths were then read by a second reader from CSIRO (R2). R2 has extensive experience in ageing southern bluefin and bigeye tunas using otoliths (using a validated method) and read the otoliths in CSIRO's albacore pilot study (Farley and Clear, 2008). R2 provided a final age estimate for 106 fish from two or three blind readings of the otoliths.

Finally, the sister otoliths from 18 of the fish were read by a third reader from 'Fish Ageing Services' (FAS) in Victoria (R3). R3 has considerable experience in ageing fish for stock assessments and routinely ages southern bluefin tuna from sectioned otoliths using the same method as R2 from CSIRO. The 18 otoliths were all from very small fish (43-56 cm) and were prepared for daily ageing by the laboratory in Qld (see daily ageing below). However, R3 was also able to provide an annual age for each otolith. Note that a 'sister' otolith is the second otolith of a pair from the same fish and both should provide the same age estimate because there are no significant differences between sister otoliths in fish species other than flatfishes (Gunn et al., 2008, Hunt, 1992). Age estimates were provided for 16 of the 18 fish.

Between reader comparisons could be made for 106 fish where R1 and R2 provided counts, and 16 otoliths where all three readers provided counts. The precision of readings was assessed using coefficient of variation (CVs), the index of average percent error (IAPE), and age bias plots (Beamish & Fournier 1981; Chang, 1982; Campana et al., 1995).

The age estimates of R1 agreed with R2 for only 14.2% of fish, although 74.5% were within 1 year (Table 3 and Table 4). The level of precision between the two readers was low (Table 4); an IAPE of 17.9% is well above the 5% value used by many laboratories as a threshold level to assess precision (Morison et al., 1998; Campana 2001). The age bias plot (Figure 2) shows that the age estimates obtained by R1 were higher on average than that obtained by R2 by ~1 year.

By contrast, the ages of R3 agreed with R2 in all of the 16 otoliths that could be compared giving a 0.0 IAPE and CV (Table 4). All fish were aged as 1 year-old by R2 and R3. R1, however, aged only 2 of these 16 fish as 1 year-olds and the other 14 fish as 2 year-olds. Given these differences, it is possible that R1 is counting an extra increment in a region of the otolith between the primordium and the first ‘true’ annual increment. This would also account for the ~1 year difference in the age-bias plot for R1 vs R2. If 1 year was subtracted from R1’s age estimates for all 106 otoliths, the IAPE and CV in the R1 vs R2 comparison dropped to 7.51% and 0.11 respectively (Table 4). These are considered relatively good levels of precision and suggest that increment counts in otoliths after age 1 are being interpreted consistently between readers.

Identifying the location of the first increment has always been difficult in tuna otoliths, and must be confirmed before increment counts can be interpreted as an estimate of age. The location of the first 1- 2 annual increments in albacore otoliths will be confirmed through daily ageing while the annual formation of the additional increments (3+) will be validated via marginal increment/edge type analysis (see validation below). At this stage, the age estimates by R2 and R3 (CSIRO and FAS) are considered more accurate, due to the experience these readers have in tuna species with validated age estimates.

Table 2. Age frequency table summarising pairwise comparisons of age estimates by R1 and R2 (n = 106). Grey indicates agreement by R1 and R2.

R2	Age	R1														Mean	Difference		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14			15	
	1	2	22	3														2.0	1.0
	2			12	2													3.1	1.2
	3				9	6												4.4	1.4
	4				2	2	2	1										5.3	1.3
	5				1	4	4	4	1									6.0	1.0
	6					1	1	4	2									6.9	0.9
	7							2	1	1								8.4	1.4
	8								3	2								8.8	0.8
	9										2							10.7	1.7
	10									1								10.3	0.3
	11										2							10.0	-1.0
	12																	14.0	2.0
	13																	-	-
	14																	-	-
	15																	14.0	-1.0

Table 3. Measures of precision of age estimates between readers.

Readers	Count	IAPE ¹	CV ²	% agreement	% agreement within 1 year
R1 vs R2	106	17.9	0.25	14.2	74.5
R1(-1yr) vs R2	106	7.51	0.11	54.7	89.6
R1 vs R3	16	29.2	0.41	12.5	100.0
R2 vs R3	16	0.0	0.00	100.0	100.0

¹ Index of average percent error (Beamish and Fournier, 1981)

² Coefficient of variation (Chang, 1982)

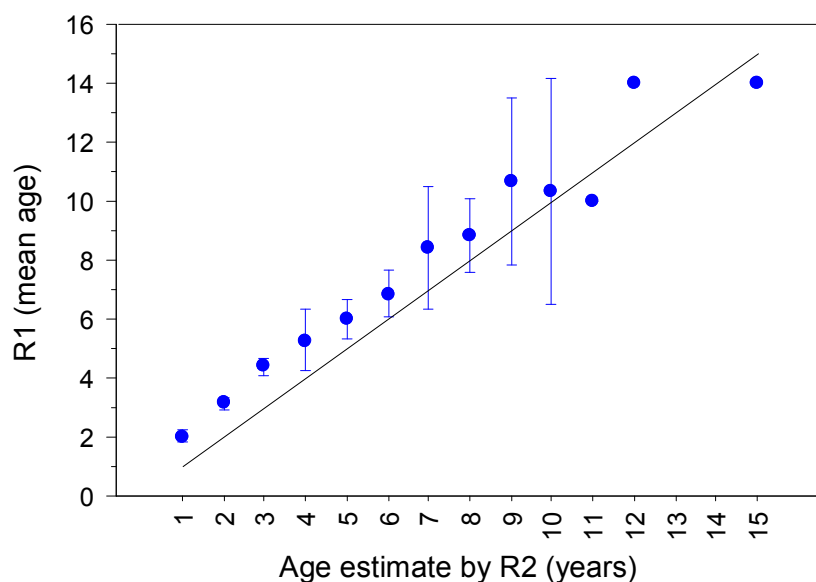


Figure 2. Age bias graph of paired age estimated by R1 and R2 (+/-95% confidence intervals).

3.2 Otolith reading (annual ageing)

Given the experience of R2, otoliths from and additional 196 albacore caught in Australia in 2009 were read and a final count assigned by this reader. Otoliths were read twice without reference to the previous reading, length of fish or date of capture. A confidence score of 0 (no pattern) to 5 (excellent) was assigned to each reading. Increment counts were obtained for 189 fish and ranged from 1 to 13. An algorithm will be developed to assign a final age to each fish based on the assumed birth date (spawning season), time of increment formation, and capture date. Figure 3 shows the length at age estimates obtained to date by R2 for albacore sampled in Australia, New Caledonia and New Zealand in 2009.

A further 58 otoliths were selected for ageing from Australia and 161 from New Caledonia/New Zealand with the aim of obtaining at least 100 age estimates for both males and females for the two (longitude) regions for 2009. Preliminary analysis indicated that age estimation of 100 individuals (or ~7 fish per age class) should provide acceptable CVs for growth parameters for albacore for each strata (sex, region and year) (Farley and Dowling, 2009). Otoliths were selected based on size of fish, sex, and month of sampling for each region so that the full size and temporal range of fish sampled by sex was selected for 2009. The otoliths were weighed and sent to FAS for sectioning. At least four serial sections will be

cut from each otolith (with one section including the primordium) and polished to 0.4 mm (Farley and Clear, 2008).

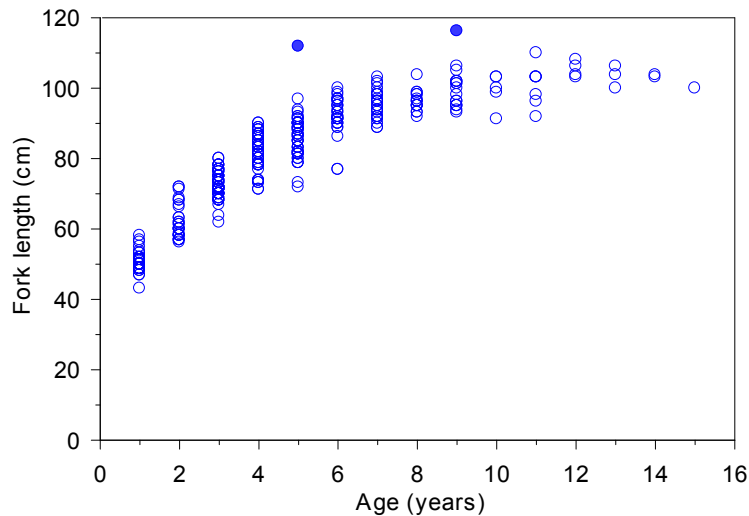


Figure 3. Length at age (increment count) estimates by R2 for albacore tuna caught in Australia, New Caledonia and New Zealand in 2009. Potential outliers have not been removed (filled circles).

3.3 Whole and sectioned otolith comparisons (annual ageing)

To determine if similar age estimates can be obtained by reading whole and sectioned otoliths, 17 sister otoliths were selected from fish that had already been aged using a sectioned otolith (in project AFMA 2006/826; Farley and Clear, 2008). The whole otolith method has proven accurate in other tropical species (e.g. Williams et al 2003), including southern bluefin tuna (Clear et al. 2000; Gunn et al, 2008) and can provide a means of cross-checking annual age estimates made using sectioned sister otoliths. The added advantage of using whole otoliths is that there is far less time required to prepare (section) the otoliths for reading.

Preparation involved using a modification of Thorogood's (1987) method: otoliths were burned on a 400°C hot plate until they turned golden brown. Although the burning accentuated the difference between opaque and translucent zones, the reader could not interpret a pattern and produce an age estimate with any confidence for more than half the samples. Age estimates from six otoliths were equivalent to, or one year different from, the estimates from the sectioned sister otoliths but for the remaining samples there was no correlation between ages estimated from the whole, burnt otoliths and ages from the sectioned sister otoliths, nor between age estimates and fish length. Because of the low confidence in readings and lack of consistency between whole and sectioned otoliths' age estimates we concluded that the whole otolith method will not be useful in this study.

3.4 Spine reading (annual ageing)

To continue the comparison of age estimates from different hardparts (see Farley and Clear, 2008), dorsal spines were also selected from 100 fish sampled from Australia for comparative age estimates. The spines were sent to FAS for sectioning following the protocols developed in Farley and Clear (2008). These spines will be read during the next 6 months of the project and age bias and precision will be examined between hard parts. Additional spines may be selected from 2009 if required.

3.5 Inter-laboratory comparisons (daily ageing)

In addition to the inter-laboratory comparison of annual age estimates, it is also important to assess the level of precision of micro-increment (assumed daily increment) counts between readers/laboratories. SPC sent 18 otoliths from small albacore (43-56 cm FL) to a laboratory in Queensland for daily ageing. One transverse section was prepared for each otolith and three micro-increment counts were provided by R1, the mean of which was calculated for use in analyses. The otoliths were then sent to FAS for a second independent reading by R3.

Between reader comparisons could be made for the 16 fish where both readers provided assumed daily age estimate (Table 5). For all otoliths, R1 obtained higher counts than R3. Counts by R1 ranged from 459-703 days (1.3 to 1.9 years) while counts by R3 ranged from 185-522 days (0.5-1.4 years). R3 noted 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. This does not, however, account for the differences in counts between readers as both were examining the same sections. It does, however, highlight the need to obtain high-quality otolith sections for micro-increment analysis.

It is not possible to determine which readers' estimates are more accurate at this stage, since we have not undertaken the age validation work yet (see below). Despite this, the back-calculated hatch dates obtained from R3 counts were more consistent with the known summer spawning season for albacore (~Oct to Apr) compared to R1 (~Mar to Jun) (Table 5) suggesting that the estimates by R3 were more accurate. The age estimates by R3 were also consistent with length-at-age estimates obtained in a previous study of South Pacific albacore (Kerandel et al., 2006) (Figure 5). Note that it is 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) 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).

Table 4. Micro-increment counts (presumed daily age) for albacore by R1 and R3. Back-calculated hatch dates are shown based on catch date and increment count.

Fish No	LCF (cm)	Catch date	R1		R3	
			Count	Hatch date*	Count	Hatch date*
1098	49	22-Jan-09	557	25-Jun-07	372	27-Dec-07
1109	56	24-Jan-09	725	10-Jan-07	434	28-Oct-07
1126	49	06-Feb-09	-	-	-	-
1129	43	10-Feb-09	459	19-Oct-07	185	20-Jul-08
1278	50	24-Feb-09	646	30-Apr-07	427	05-Dec-07
1280	52	24-Feb-09	680	27-Mar-07	419	13-Dec-07
1281	49	24-Feb-09	582	03-Jul-07	340	01-Mar-08
1282	51	24-Feb-09	635	10-May-07	472	21-Oct-07
1283	50	24-Feb-09	691	16-Mar-07	522	01-Sep-07
1284	54	24-Feb-09	722	13-Feb-07	-	-
1285	51	24-Feb-09	652	23-Apr-07	445	17-Nov-07
1289	51	26-Feb-09	621	26-May-07	418	16-Dec-07
1298	51	02-Mar-09	600	20-Jun-07	308	08-Apr-08
1299	52	02-Mar-09	609	11-Jun-07	328	19-Mar-08
1301	52	03-Mar-09	616	05-Jun-07	323	25-Mar-08
1303	53	03-Mar-09	703	11-Mar-07	385	23-Jan-08
1304	47	03-Mar-09	692	22-Mar-07	433	06-Dec-07
1305	52	03-Mar-09	690	23-Mar-07	464	05-Nov-07

* Hatch dates were calculated using the catch date and the estimated age minus 20 days since ~15-20 zones were estimated to be missing from the counts.

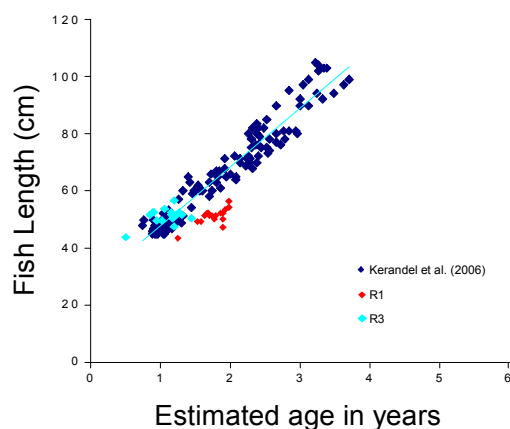


Figure 4. Comparison of estimated daily age (converted to annual age) obtained by R1 and R3 compared to that obtained by Kerandel et al. (2006) for albacore in the South Pacific.

3.6 Age validation

Mark-recapture experiment (direct validation)

One of the best ways to validate the period of increment formation (both daily and annual) is through a mark-recapture experiment using a dye to mark the hardparts, such as otoliths and spines, at the time of release. SPC is currently undertaking an age validation experiment for albacore using oxytetracycline (OTC) to mark the hardparts (Williams et al., 2009). In early 2010, a 'marked' albacore was recaptured in New Zealand, and the hardparts subsequently sampled. One otolith and the fin spine have been sent to FAS for sectioning and blind reading.

For age to be validated, the number of micro-increments counted after the OTC mark must equal the known time at liberty.

Location of the first annulus (indirect validation)

Micro-increment analysis can also be used to confirm the position of the first 1-2 annual increments (opaque growth zones) in otoliths since the first annual increment should occur on or before the 365th daily increment, and the second annual increment on or before the 730th daily increment. Assuming that the micro-increment counts by R3 (above) represent daily age, measurements were made from the first inflection point on the otolith to the 365th increment (age 1; Y1) and to the edge of the otolith. These measurements were compared with the locations of the first (annual) opaque zone in the corresponding sister otolith sectioned for annual ageing. 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.

These comparisons confirmed that the first annual increment was being successfully identified in all 16 otoliths where measurements were available. 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 forms just prior to the 1st birthday. An additional 30 otoliths have been selected for micro-increment analysis to continue this indirect validation. The otoliths will be prepared by FAS and read by R3.

Time of increment formation (indirect validation)

When otoliths were read for annual age estimation, the last (outer) band was assigned an edge type category to describe its appearance as new (opaque), intermediate (translucent zone < 2/3 complete) or wide (translucent zone > 2/3 complete). This was only undertaken for otoliths with a reading confidence score of 3 to 5 since the terminal edge of some otoliths is not clear enough to be sure of the terminal edge type. When sufficient monthly samples of specific age classes have been obtained, edge type analysis will be undertaken to determine if and when increments form annually. Marginal increment analysis will also be undertaken if possible.

5. Histological analysis of ovaries

Histological sections prepared from 197 ovaries were read and staged based on the criteria outlined in Farley and Clear (2008). The ovaries were from fish >70 cm FL sampled in Australia, New Caledonia and New Zealand. The smallest mature female sampled was 79 cm FL, while the (very) preliminary analysis predicted that the length at 50% maturity based on pooled data was 83.0 cm for fish caught in Australia, and 83.7 cm for fish caught in New Caledonia/New Zealand.

An additional 220 ovary samples have been sent for histological preparation. These were sampled predominantly from Australia, New Caledonia, Fiji, and French Polynesia. Again, the ovaries selected were from females >70 cm fork length. This size range was selected as it

encompass immature to 100% maturity which is important for examining reproductive characteristics such as spawning time/area, spawning frequency, and estimating size/age-at-maturity.

Blood plasma samples collected from albacore in New Zealand will be analysed to determine if steroid hormone levels in the blood can be useful for estimating reproductive parameters such as maturity and spawning frequency.

6. Length-weight relationship

The relationship between fork length and weight for albacore sampled in Australia and New Zealand is shown in Figure 5. Almost all fish >100 cm FL sampled were male; a dominance of males in length classes greater than ~ 90 cm and an absence of females >100 cm has been reported in other studies and may be due to differential mortality of sexes and/or differential growth rate after maturity (Bard, 1981; see Alonso et al. 2005).

Length-weight parameters were estimated using least-square linear regression of log-transformed data for all fish and by sex. Analysis of covariance found a significant difference in the length-weight relationship between sexes ($F = 43.38$; $P < 0.0001$) where females were heavier on average for their length compared to males. For example, females are 20g heavier on average at 50cm, and 600 g heavier at 100 cm. A significant difference still existed when fish >100 cm FL were removed from the analysis ($F = 31.70$; $P < 0.0001$). This difference may simply be due to the difference in the weight of gonads (and attached fat body) between the sexes and will be investigated further.

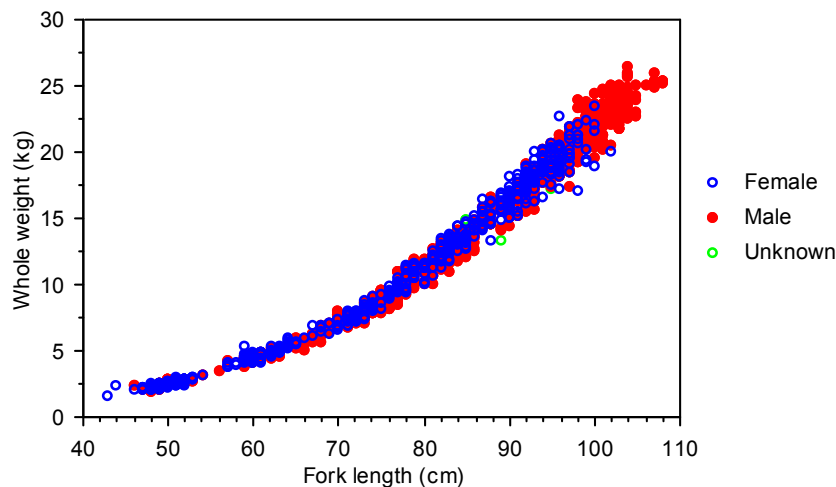


Figure 5. Plot of fork length (cm) to weight (kg) for all albacore sampled in Australia and New Zealand since 2007. $n=1263$.

Table 5. Regression coefficients for length-weight (cm-kg) relationships for albacore sampled in Australia and New Zealand (since 2007). 'Combined' includes males, females and unknown sex.

Sex	a (intercept)	b (slope)	r^2	n
Female	9.18×10^{-6}	3.196	0.993	537
Male	9.828×10^{-6}	3.176	0.995	698
Combined	9.54×10^{-6}	3.175	0.994	1263

7. Future work

The priority for the next 12 months is to conclude the biological sampling of albacore and continue the analysis of hardparts and gonads. Sampling of albacore in Australia will finish at the end of 2010. Collection of biological samples from the PICTs will also conclude at the end of 2010. The focus of the remaining months will be to obtain samples from areas where little or no samples are available from to date, including the Cook Islands. Additional samples from Tonga will be collected during upcoming phases of SPC albacore tagging in the Tongan EEZ in 2010.

The analysis of hardparts and gonads will continue for at least the next 12 months, and biological parameters will be delivered to stock assessment and harvest strategy scientists by the end of 2011.

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8. References

- Alonso, C., Arrizabalaga, H., and Restrepo, V. R. 2005. Contribution of a chapter on albacore tuna for the revised ICCAT field manual. Collective Volume of Scientific Papers International Commission for the Conservation of Atlantic Tunas 58, 1646-1669.
- Bard, F. X.(1981). Le thon germon (*Thunnus alalunga*) de l'Océan Atlantique. PhD Thesis presented at the University of Paris, 333p.
- Beamish, R., and Fournier, D.A. 1981. A method for comparing the precision of a set of age determinations. Journal of the Fisheries Research Board of Canada. 36: 1395–1400
- Brothers, E. B., Prince, E. D., Lee, D W. 1983. Age and growth of young-of-the-year bluefin tuna, *Thunnus thynnus*, from otolith microstructure. NOAA tech. Rep. NMFS 8: 49-59
- Campana, S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59: 197-242.
- Farley, J. and Clear, N. 2008. Albacore tuna: investigation of size monitoring, age composition, and spawning activity in the ETBF. Final report to the Australian Fisheries Management Authority, Project 2006/826, 66p.

Farley, J. and Dowling, N. 2009. Industry based size-monitoring and data collection program for albacore tuna in the ETBF. Final report to the Fisheries Research and Development Corporation, Tactical Research Fund Project 2008/075, 118p.

Gunn, J. S.; Clear, N. P.; Carter, T. I.; Rees, A. J.; Stanley, C. A.; Farley, J. H. and Kalish, J. M. 2008. Age and growth in southern bluefin tuna, *Thunnus maccoyii* (Castelnau): Direct estimation from otoliths, scales and vertebrae. Fisheries Research. 2008. 92(2-3):207-220
ISSN: 0165-7836.

Hunt, J.J. 1992. Morphological characteristics of otoliths for selected fish in the Northwest Atlantic. J. Northw. Atl. Fish. Sci., Vol. 13: 63–75.

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

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

Kerandel, J-A., Leroy, B., and Kirby, D.S. 2006. Age and growth of albacore by otolith analysis. SPC Oceanic Fisheries Programme Internal Report, Secretariat of the Pacific Community, Noumea, November 2006.

Morison, A. K., Robertson, S. G. & Smith, D. G. 1998. An integrated system for production fish aging: image analysis and quality assurance. North American Journal of Fisheries Management 18: 587–598.

Radtke, R.L. 1984. Scanning electron microscope evidence for yearly growth zones in giant bluefin tuna, *Thunnus thynnus*, otoliths from daily increment. Fish Bull 82: 434-440.

Thorogood, J. 1987. Age and growth rate determination of southern bluefin tuna, *Thunnus maccoyii*, using otolith banding. J. Fish. Biol. 30 : 7-14.

Williams, A., Nicol, S., Hampton, J., Harley, S. and Hoyle, S. 2009. South Pacific Albacore Tagging Project: 2009 Summary Report. WCPFC-SC5-2009/GN-IP-16.

Williams, A.J., Davies, C.R., Mapstone, B.D., and Russ, G.R. 2003. Scales of spatial variation in demography of a large coral-reef fish—an exception to the typical model? Fisheries Bulletin 101: 673-683.