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BIGEYE TUNA AGE, GROWTH AND REPRODUCTIVE BIOLOGY (PROJECT 35)

WCPFC-SC7-2011/SA- WP -01
Revision 1 (3 August 2011)

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Executive Summary

The Fifth Regular Session of the Commission in December 2008 endorsed the "Comprehensive Research Plan on Pacific-wide Bigeye Growth and Reproductive Biology" (WCPFC SC4 BI-WP-7). The research plan comprises an initial pilot phase and a full implementation phase. Project 35 (Bigeye growth and reproductive biology) of the WCPFC Scientific Committee is the implementation of the pilot study. Its purpose is to determine the sampling requirements for implementation of a Pacific-wide study and the feasibility of sampling from longline and purse-seine vessels. Region 3 of the bigeye stock assessment was prioritised for implementation of the pilot study. In 2010, the WCPFC Scientific Committee further requested that the analyses component of Project 35 include three runs of the 2010 bigeye stock assessment model; specifically i) the use of the growth curve estimated from the pilot study; ii) the use of the maturity ogive estimated from the pilot study; and iii) a combination of (i) and (ii); to evaluate the sensitivity of the bigeye stock assessment to these new estimates.

<u>Primary results</u>

1. The maturity ogive estimated for the pilot study is consistent with other estimates for the WCPO from equatorial waters but significantly different to that estimated for the EPO. Significant changes in the maturation dynamics of bigeye tuna are most likely to occur east of 130°W longitude.
2. The spawning biomass and fishing mortality reference points in the 2011 bigeye stock assessment were different when the pilot study maturity ogive was used rather than the EPO ogive. The estimates were more optimistic however the magnitude of improvement was <5%. This level of sensitivity was also noted in the analyses performed in the 2008 bigeye stock assessment (Hoyle and Nicol 2008).
3. The correspondence between the growth curve estimated from the pilot study and that generated from MFCL for region 3 provides preliminary information that the length-based growth estimates from MFCL are consistent with those derived from otoliths.
4. There is evidence of latitudinal and longitudinal variation in growth within the WCPO.
5. The magnitude of difference between the estimate of growth from the pilot study and the estimate used in the 2011 assessment base case significantly influenced the estimate of the fishing mortality reference point.

<u>Key Recommendations</u>

1. The use of the EPO maturity ogive does not seem appropriate for the WCPO bigeye stock. It is recommended that future assessments use a maturity ogive that incorporates data from the WCPO.
2. It is recommended that further refining the spatial variation in the maturity ogive is a lower priority for the full Pacific-wide study.
3. Variation in the maturation dynamics of bigeye tuna between the WCPO and EPO, however, will have important consequences for the model structure of future Pacific-wide bigeye tuna stock assessments.

4. The implementation of the Pacific-wide study on growth is recommended. It will provide primary data for developing an understanding of the spatial variation in growth and for future structuring of the WCPO bigeye stock assessment. A more comprehensive understanding of the biological processes that result in spatial variation in growth would also significantly benefit future model structures for bigeye.

5. The coarse spatial stratification of 32° longitude X 20° latitude for the Pacific-wide study as specified in the research plan (8 strata in total for the WCPO) is recommended with 300 otoliths per strata as the minimum sample size required.

6. There is considerably less cost associated with annual ageing of otoliths in comparison to daily ageing. Given the close correspondence in ages obtained between three otolith-based methods compared, it is recommended that annual ageing be adopted for the full Pacific-wide study.

7. The training of PIRFO observers will need to continue if they are to assist with the collection of samples for the Pacific wide study. It is recommended that the Scientific Committee and Commission provide guidance to PIRFO programs on the priorities for biological sampling over the short to medium term (ie. 1-5 years) so that training and logistical support for samples can be included in PIRFO plans and budgets.

8. The most cost effective method for implementation of the Pacific wide study would utlise all existing regional and national observer programs operating in the WCPO. To realize this opportunity, a coordinator in each of these programs needs to be identified. The successful implementation of the Pacific-wide study will also require a level of central coordination to ensure adequate sampling of each strata. It is recommended that the WCPFC secretariat undertake this role (duties of existing staff or through its science provider).

6. A central repository to store and archive samples is required to ensure access to the samples and data for all WCPFC members.

7. In addition to the inkind contribution by WCPFC members through observer program participation, the budget for implementing the WCPO component of the Pacific wide study is USD90,000 per year for 3 years. This budget is based on USD50,000 per year for sampling support to developing states and participating territories and project coordination by WCPFC Secretariat, USD15,000 for travel & freight, USD 5,000 for sample curation and USD20,000 for analyses of samples. All other costs (eg observer participation) are assumed to be contributions from member countries.

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1.0 Project Acknowledgements

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2.0 Introduction

The Fifth Regular Session of the Commission in December 2008 endorsed the "Comprehensive Research Plan on Pacific-wide Bigeye Growth and Reproductive Biology" (WCPFC SC4 BI-WP-7, Nicol et al. 2008). The goal of this Research Plan is to improve stock assessment and management of bigeye tuna in the Pacific Ocean. The specific objectives are:

1. To obtain data that will contribute to, and reduce uncertainty in, the maturity schedule used in stock assessment models, over the equatorial and sub-equatorial range of bigeye.
2. To obtain comprehensive information on the growth rate of bigeye and the spatial and seasonal variation expected in this rate.
3. To obtain information on bigeye fecundity, and the influence of age and size

The research plan comprises an initial pilot phase and a full implementation phase. Project 35 (Bigeye growth and reproductive biology) of the WCPFC Scientific Committee is the implementation of the pilot study. Its purpose is to determine the sampling requirements for implementation of a Pacific-wide study and the feasibility of sampling from longline and purse-seine vessels. Region 3 of the bigeye stock assessment was prioritised for implementation of the pilot study. Due to difficulties in obtaining a sufficient number of samples within the time frame of the pilot study, the collection of samples was extended from the 170°E longitude boundary of region 3 to the 180° meridian to include the opportunity for sample collection from the Marshall Islands EEZ. The area from 170°E to 180° is within Region 4 of the bigeye stock assessment.

In 2010, the WCPFC Scientific Committee further requested that the analyses component of Project 35 include three runs of the 2010 bigeye stock assessment model; specifically i) the use of the growth curve estimated from the pilot study; ii) the use of the maturity ogive estimated from the pilot study; and iii) a combination of (i) and (ii); to evaluate the sensitivity of the bigeye stock assessment to these new estimates in order to assist the Scientific Committee in determining the priority for full implementation of the Research Plan.

3.0 Sampling Feasibility

Gonads and sagittal otoliths were sampled from bigeye caught primarily within the EEZs of Palau, Federated States of Micronesia and Marshall Islands in 2009, 2010 and 2011. Over the period of the pilot study, three approaches to collecting samples were implemented:

1. Sampling by at-sea observers in combination with port samplers;
2. Sampling by crew members on longline vessels in combination with port samplers;
3. Sampling by fisheries technicians at sea and in port.

<u>At Sea Observers</u>

There was excellent cooperation by fishing vessels for observers to undertake biological sampling as part of their duties. To avoid any lack of cooperation during the full Pacific wide phase, it is advised that vessels be notified that biological sampling will be conducted during the trip as part of the "Observer Placement Meeting" that is undertaken prior to departure between the delegate from the observer program and vessel captain. The availability of adequately skilled and trained observers was identified as limiting the capacity to implement biological sampling on a routine basis at the commencement of the project. To increase the capacity for biological sampling, training materials have been developed and distributed to those conducting observer training courses. Training of observers has been undertaken in conjunction with the Pacific Islands Regional Fisheries Observer Program (PIRFO) schooling activities since the pilot project commenced and training is now included formally in most PIRFO training courses. Training consists of instruction and practical sessions in extraction of otoliths (using a variety of methods), gonad identification, sex identification, storage of samples and data recording. The training is typically conducted over a 2-3 day period and includes presentations on the scientific use of biological samples and their purpose in fisheries management. In addition, observers were also instructed in the collection of other biological samples (genetic, stomach, liver, muscle) so that they are able to contribute to other WCPFC-endorsed activities. In addition, a specific workshop was conducted to provide competency-based training to the PIRFO Trainers.

Biological sampling standards have also been developed for the PIRFO to assist with establishing competencies and capacity within the observer programs of the WCPO. Similar standards should be considered for adoption by other observer programs operating in the region to establish the capacity for Pacific-wide sampling. Collection of biological samples at sea also requires that observers are issued with appropriate equipment for extraction and storage. Equipment kits are likely to vary between USD100-USD200. During the pilot study no issues were encountered concerning the storage of biological samples on vessels (including the freezing of gonads). Storage of frozen samples after the vessel returns to port was more problematic and an important consideration for implementing a Pacific-wide study will be provisioning relevant authorities in all ports where observers disembark with freezer capacity for biological samples that are preserved frozen.

For purse-seine vessels, sampling by PIRFO at-sea observers to collect gonads and otoliths was effective with observers noting that there was sufficient time, space on the vessel and cooperation from the vessel captain and crew to undertake biological sampling and assist with storage of samples. Longline observer coverage over the pilot study has been extremely low and it was not possible to collect many samples from longliners using PIRFO observers. Collection of otoliths and matching gonads from fish that are caught on vessels supplying the fresh fish market can be problematic as vessels/companies prefer otolith extraction to occur after the grading of the fish in port. As these fish are dressed at sea, this creates the potential problem of not being able to match gonads (or sex determination) with otoliths from the same individual. Cable tagging fish potentially resolves this issue. This method places a non-damaging, easily removable and uniquely numbered tag around the lip of the fish (Figure 1). The tag includes disposable labels (with the tag number printed on it) that can be used to label biological samples collected on the vessel. When the individual is landed and is dressed on the vessel, the tag can be place around the lip, the gonad can be collected and the disposable tag ripped off and placed in the bag with the gonad. Otoliths can then be extracted in port and the cable tag included with the stored otoliths. The gonads and otoliths can then be matched through the unique sample number.

A port coordinator is essential for this type of sampling to be effective. The vessel unloading for fresh fish operations in Palau, Pohnpei and Marshall Islands are extremely efficient with individuals graded for quality and then either packaged for transport to market or sent to adjoining loining operations occurring very quickly. Consequently, identification of fish that require otolith extraction needs to occur immediately and arrangements for extraction organized. It is worth noting that in each of these ports we were not able to obtain access to individuals designated for transport to fresh fish markets. The only option for extraction of otoliths was after auction in Japan, where a fee of USD50 per fish is required to compensate the Japanese buyers. For fish to be loined locally, it is critical that the port coordinator contact the loiner immediately. We observed on numerous occasions that the otoliths can be lost during loining unless the loiners are made aware that the fish needs to be butchered in a particular manner to retain the otoliths with the fish head. The inability to sample individuals for the fresh fish market resulted in a non random sample for the pilot study for the domestic longline fisheries of Palau, FSM and Marshall Islands as fish graded A or B sashimi quality were not sampled.

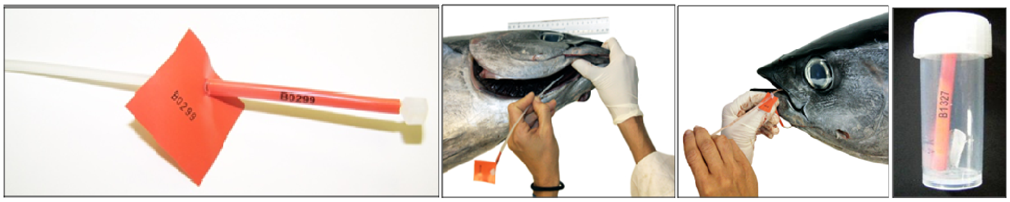

Figure 1. Left Panel. Uniquely labelled cable tie tag with detachable label for gonads. Middle Panels. Placement of tag around lip of caught tuna. Right Panel. Otoliths stored with cable tie number.

We did not collect any samples from deep-freezer longline vessels as the operations of these vessels was largely outside of the spatial domain specified for the pilot study. Many of the issues raised above are unlikely to be relevant for these vessels. The experience of Japanese, Taiwanese and New Zealand observers (E. Chang, S. Harley, N. Miyabe pers. comm.), who have extracted bluefin otoliths from these boats, is that this activity can be undertaken during the fish dressing operations on the vessel, as any concerns about head rigidity impacting sale price are minor as the fish are immediately deep frozen.

<u>Sampling by crew members</u>

Due to the low PIRFO coverage on longline vessels since 2009, the Luen Thai Fishing Venture offered assistance through collection of samples by crew members on their vessels. The participation level, however, was low and although instructed in the cable tie tagging method for obtaining matching gonads and otoliths from individuals and the requirements for storage, the quality of samples was poor. The crew is typically occupied with fishing activities during hauling which most likely explains the low participation rate. An option of providing payment to the crew for the samples was explored, however this did not increase participation rate.

<u>Sampling by fisheries technicians</u>

This was the most successful option implemented in the pilot study in terms of quantity and quality of samples and associated catch data. Under contract to the pilot project, a senior observer from Marshall Islands undertook dedicated trips for biological sampling in FSM and Marshall Islands. On return to port, trained technicians were on site to extract otoliths. For collection of biological samples from the domestic longliners, this is will be an efficient and effective method.

<u>Transport of Frozen Samples</u>

The transport of gonad samples from the port of unloading to the laboratory that undertakes histology is a significant issue. The costs incurred through freighting companies can be considerable after agent fees are included for importation into countries with suitable histology facilities. Obtaining importation permits for large quantities of biological material may also prove costly. Transfer as accompanied luggage of fisheries officers as they attend various meeting throughout the Pacific region is an option, however airline fees for excess luggage are often equivalent to freight and agent fees. This method of transport can be compromised if any defrosting of samples occurs.

4.0 Reproductive Biology

A total of 282 gonads have been collected since March 2009 for the pilot study (Table 1, Figure 2). The length distribution of the bigeye sampled is provided in Table 2. Age estimates for maturity are not presented as the number of samples with matching otoliths and gonads was insufficient for reliable analyses (55 female, 65 male, Table 2).

Table 1. Number of bigeye gonads collected by EEZ and sex

EEZ	Female	Male	Total
FSM	52	58	110
RMI	61	72	133
Other	19	20	39
Grand Total	132	150	282

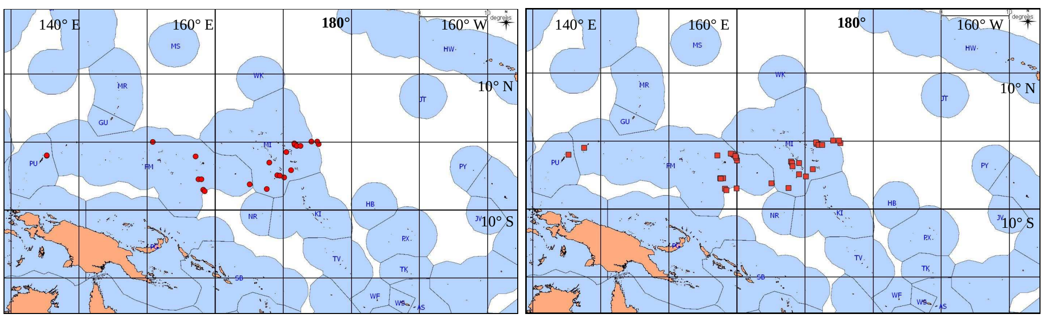

Figure 2. The spatial distribution of gonad samples collected in the pilot study including FSM, RMI and Palau. Left panel is male samples and right panel is female samples.

All gonads collected were weighed to the nearest 0.1 g if sampled whole, the sex confirmed and a subsample preserved in 10% buffered formalin. Histological sections were prepared for gonads from fish >80 cm UF – the minimum size recommended by Schaefer et al. (2005) for maturity estimation. All fish <80 cm UF were considered immature. To date, only the ovaries have been classified into one of 6 reproductive states (Table 3) depending on the oocytes, atretic state and postovulatory follicle class present in histological sections. The classification scheme is adapted from those developed by Schaefer (1998), Farley and Davis (1998) and Farley and Clear (2008). Females in stages 3-6 are classed as mature. The testes have not been classified because the histological sections prepared from frozen material were not suitable for staging adequately. Frozen ovary material is suitable for staging and has been undertaken successfully in other tunas and billfish.

Length at 50% maturity was calculated using a logistic regression on 5 cm length increments:

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

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

We did not calculate spawning frequency as 29 out of 100 ovaries had hydrated oocytes, and postovulatory follicles (POF) are difficult to detect when these oocytes are present. We considered that any estimate would be inaccurate due to the reduced sample size once the ovaries with hydrated oocytes were removed from the analyses. The time when the fish were landed alive was not collected precluding the use of the hydrated oocytes method.

Table 2. Length distribution of bigeye sampled for gonads. Note numbers do not include otoliths if they had been sampled but were not suitable for ageing (eg broken).

	Number of samples			
	Female		**Male**	
Size class (cm UF)	**Gonad only**	**Gonad & otolith**	**Gonad only**	**Gonad & otolith**
31-40	3		4	
41-50	1	1	2	
51-60	3	5	2	4
61-70	2	5		4
71-80		10	2	18
81-90	2	5	1	5
91-100	2	3	2	1
101-110	7	5	18	4
111-120	19	12	16	12
121-130	16	4	17	7
131-140	19	5	10	4
141-150	2		9	2
151-160	1		1	3
161-170				
171-180			1	
181-190				1
Total	77	55	85	65

The relationship between ovary weight and length by reproductive state for females >80 cm FL (M= mature and I =immature) is presented in Figure 3 and the length frequency by activity classification for females >80 cm FL in Figure 4. The number of females and percent mature by length class is presented in Table 4 and proportion mature in Figure 5.

There is some uncertainty whether individuals classified as Stage 7 (Table 3) are mature and regenerating or immature. The (L_{50}) estimated was 105.9 cm when these individuals were considered mature and was 107.8 cm when these individuals were considered immature. The smallest mature fish was 101 cm (regenerating), the smallest actively spawning female was 104 cm, and largest immature female was 118 cm. The results are consistent with previous estimates of the maturity ogive for bigeye in the western and central Pacific Ocean (Table 5). The high proportion (50%) of samples that were classified as actively spawning which is most likely associated with the sea surface temperatures exceeding 25°C and shallow fishing depths.

Table 3. Number of females by maturity classification. POF = Postovulatory follicle.

Stage	Maturity	Activity	Classification	Advanced oocyte and POF stage	Atresia of advanced yolked oocytes	Additional maturity markers[6]	Count
1	Immature	Inactive	Immature	Unyolked, no POFs	No alpha or beta atresia	None	16
2	Immature	Inactive	Developing	Early yolked, no POFs,	No alpha or beta atresia	None	5
3	Mature	Active	Spawning capable[7]	Advanced yolked, no POFs,	<50% alpha atresia, beta atresia may be present	May be present	14
4	Mature	Active	Actively spawning	Migratory nucleus or hydrated and/or POF's	<50% alpha atresia, beta atresia may be present	May be present	50
5	Mature	Inactive	Regressing - potentially reproductive	Advanced yolked, no POFs	>50% alpha atresia, beta atresia present	May be present	3
6a	Mature	Inactive	Regressed (1)	Early yolked no POFs	100% alpha atresia, beta atresia present	May be present	2
6b	Mature	Inactive	Regressed (2)	Early yolked no POFs	No alpha atresia, beta & delta atresia present	May be present	5
7	Mature	Inactive	Regenerating	Unyolked or early yolked, no POFs	No alpha or beta atresia. Gamma/delta atresia may be present	Present	5
Total							100

[6] Maturity markers are structures remaining in the ovary that indicate the fish has either spawned (residual hydrated oocytes) or has produced advanced oocytes (follicular cysts) in the past. See Farley et al. (2011) for more details.

[7] Some of the 'spawning capable' females may be actively spawning but the POFs could not be detected due to reduced tissue quality. The tissue quality did not affect the ability to stage oocytes, atresia or maturity markers.

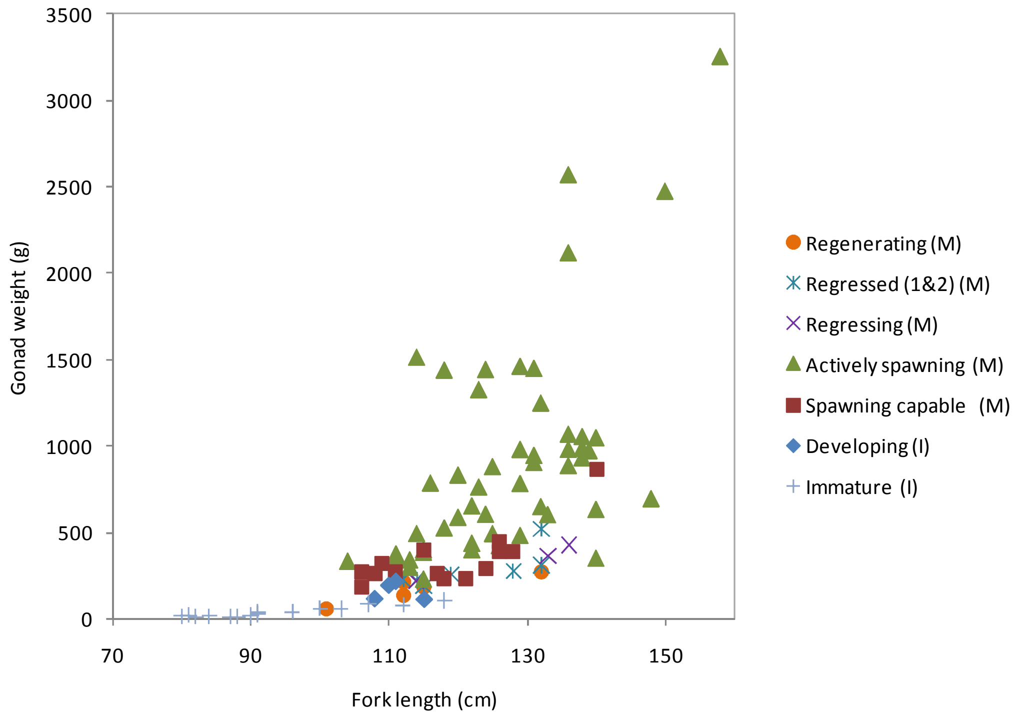

Figure 3. The relationship between ovary weight and length by reproductive state for females >80 cm FL (M= mature and I =immature).

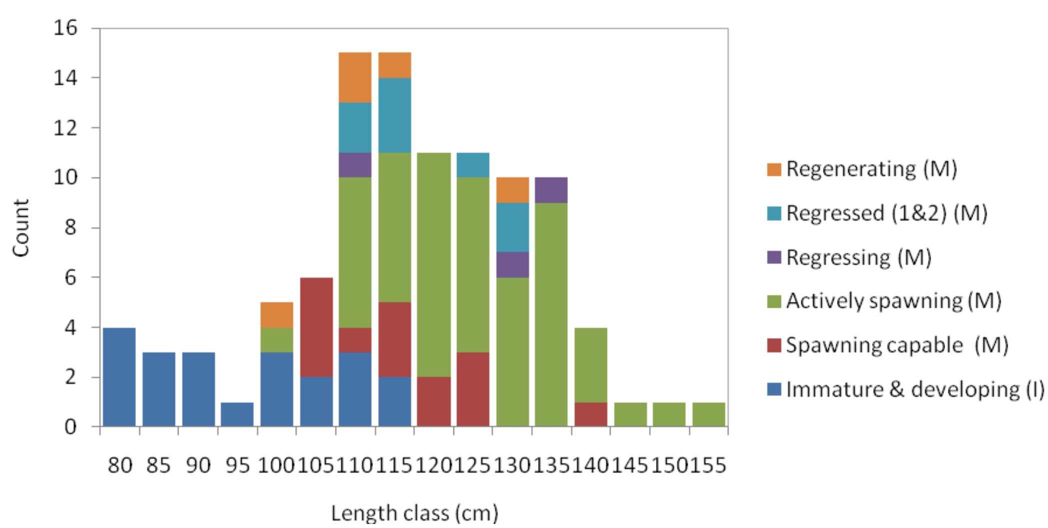

Figure 4. Length frequency by activity classification for females >80 cm FL.

Table 4. Number of females and percent mature by 5 cm length class. 'Mature 1' uses the classification scheme from Table 3. 'Mature 2' classifies all Stage 7 females as immature

Length class (cm)	N	% Mature 1	% Mature 2
80	4	0.0	0.0
85	3	0.0	0.0
90	3	0.0	0.0
95	1	0.0	0.0
100	5	40.0	20.0
105	6	66.7	66.7
110	15	80.0	66.7
115	14	85.7	78.6
120	11	100.0	100.0
125	11	100.0	100.0
130	10	100.0	90.0
135	10	100.0	100.0
140	4	100.0	100.0
145	1	100.0	100.0
150	1	100.0	100.0
155	1	100.0	100.0
Total	**100**		

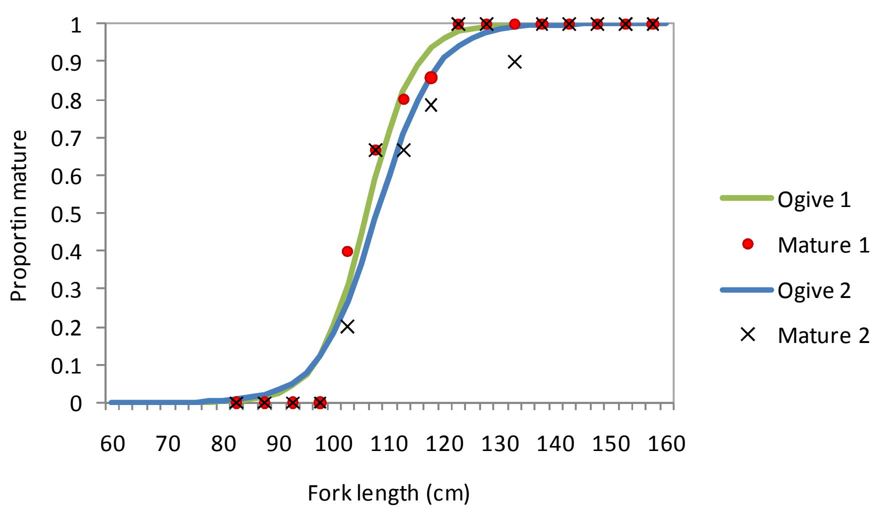

Figure 5. Proportion of females mature by 5 cm length class. 'Mature 1' uses the classification scheme from Table 3 (L_{50} = 105.9 cm). 'Mature 2' classifies all Stage 7 females as immature (L_{50} = 107.8 cm).

Table 5. Estimates of maturity at length/age for female bigeye for the Pacific Ocean.

Study	Method	Location	Estimate
Farley et al. 2003; 2006	Macroscopic n = 635 ovaries	Australia (east coast)	L_{min}=80 cm FL L_{50}= 102.4 cm FL females
Sun et al. 2002; 2006; unpubl. data	Histological n= 380 gonads	Philippines	L_{min}=99.7 cm FL females L_{50}=105 cm FL females
Yuen 1955 (Secondary citation used)		Marshall Is Line Is (Kiribati) Hawaiian Is	L_{min}= 90-100 cm FL
Zhu et al. 2010	Histological n = 429 ovaries	Central Pacific	L_{min}=94 cm FL L_{50}= 107.8 cm FL females
Schaefer et al. 2005; 2006	Histological n = 683 ovaries	EPO	L_{min}=102 cm FL females L_{50}=135 cm FL females

5.0 Age and Growth

A total of 313 otoliths have been collected since March 2009 for the pilot study (Table 6, Figure 6). The length distribution of the bigeye sampled is provided in Table 6. Three analyses have been undertaken:

1. Estimation of the growth curve for region 3 using the data derived from annual increment counts of the otoliths collected during the pilot study to use in the sensitivity analyses requested at SC6. Analyses to test for differences in growth between sex was not undertaken as the number of samples with matching otoliths and gonads was insufficient for reliable analyses (55 female, 65 male, Table 2).
2. Analyses to test for regional growth differences. In this component of the analyses we have compared the Region 3 otolith derived growth curve with that estimated for each stock assessment region using MFCL and that derived from otoliths data from the Coral Sea (Farley et al. 2006; Region 5 of the stock assessment model)
3. Comparison of the methods used to estimate the age of bigeye tuna using increment counts of sectioned otoliths. In the Pacific Ocean, daily and annual increments have been used to age sectioned otoliths. In addition, two different approaches to sectioning otoliths have also been compared. In the eastern Pacific Ocean, counts of daily increments along a longitudinal cross-section of the otolith has been used on the assumption that its greater length allows older individuals (4 to 5 years old) to be aged more precisely (ie. the daily increments are further apart and thus easier to read). In the western and central Pacific Ocean, daily increments along a transverse cross-section of the otolith have been used. The shorter transverse section only allows fish to be aged to a maximum of 3 to 4 years. Annual counts of increments have also been applied to transverse cross-sections. This method has allowed individuals to be aged up to 16 years (Farley et al. 2006). Each method has been validated through marginal increment analyses, and the ageing of externally tagged and chemically marked fish (ie known aged fish). We compared

the age estimates between both daily methods and from the annual method to determine whether comparison between age data derived from each method is valid.

Estimation of bigeye age using annual increments

Of the otoliths sampled, 289 were selected from those sampled in region 3 for age estimation using counts of opaque increments in transverse sections. It is assumed that one opaque increment form annually in otoliths as has been validated for bigeye tuna (Farley et al. 2003; 2006). Sagittal otoliths were removed, cleaned of any residual material, and stored dry. Otoliths were sectioned and embedded in epoxy resin and cut transversely through the primordium with a diamond-tipped blade on a low-speed saw to produce a thin section of 300-400 µm. Sections were mounted on glass slides using Crystal BondTM adhesive. The number of visible opaque growth zones was counted along the ventral 'long' arm of each otolith using the techniques developed for bigeye tuna (Farley et al., 2006). A confidence score of 1 (poor) to 5 (excellent) was assigned to each reading. Otoliths were read twice 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 von Bertalanffy growth function was fitted to the length-at-age data using the equation:

$$L_t = L_\infty(1 - e^{-k(t-t_0)})$$

where L_t is the fork length (cm) at age t, L_∞ is the theoretical maximum fork length, k is the rate at which L_∞ is approached and t_0 is the theoretical age (years) at zero length. The equation was fitted by non-linear least square regression of length on age. For

Annual increment counts could not be observed in 13 of the 189 otoliths examined. The estimated length at age relationship for the 289 otoliths that could be aged is presented in Figure 7. The age frequency distribution is presented in Figure 8 and the von Bertalanffy growth parameters were t_0=-0.96, k =0.354, L_∞=156 cm.

Regional growth differences

To examine spatial variation in growth we compared the MFCL von Bertalanffy curves for regions 1-6 and base case (all extracted from the 2011 stock assessment; Table 7, Figure 9a) together with the otolith derived curve for region 3 and a second growth curve for Region 5 (estimated from otoliths, Farley et al. 2006; Table 7). For the otoliths data we calculated the von Bertalanffy growth with a quarter year time step for comparison with estimates from MFCL. Examination of the growth curves from the 6 MFCL regions indicates significant longitudinal and latitudinal variation on growth (Figure 9a). The two growth curves estimated for region 3 were similar (Figure 9b, Table 7) as were the growth curves estimated for region 4 and the base case (Figure 9c, Table 7) and the curves estimated for region 5 and 6 by MFCL (Table 7). The Region 5 curve estimated from otoliths differed to that estimated by MFCL (Figure 9d, Table 7) however this difference can partially be explained by the absence of fish below 70 cm in the otolith data set.

Table 6. Number of bigeye otoliths collected by EEZ and length class. F= female, M= male, U= Unknown

Number of otolith samples													
	FSM			RMI			Palau			Other			Total
Size class (cm UF)	**F**	**M**	**U**	**F**	**M**	**U**	**F**	**M**	**U**	**F**	**M**	**U**	
31-40										1			1
41-50													
51-60	3		2	2	1					2	3		13
61-70	1	1			3					3	3		11
71-80	8	8			6					2	4		28
81-90	2			2	3					1			8
91-100	1		1	1	1					1			5
101-110	1		10	3	4	2			1				21
111-120	3		28	9	12	5			4				61
121-130	1	2	30	3	5	4			12				57
131-140	2		22	3	4	8			17				56
141-150			12		2	7			19			1	41
151-160		1	2		2				2				7
161-170									1				1
171-180			2										2
181-190					1								1
Total	22	12	109	23	44	26			56	10	10	1	313

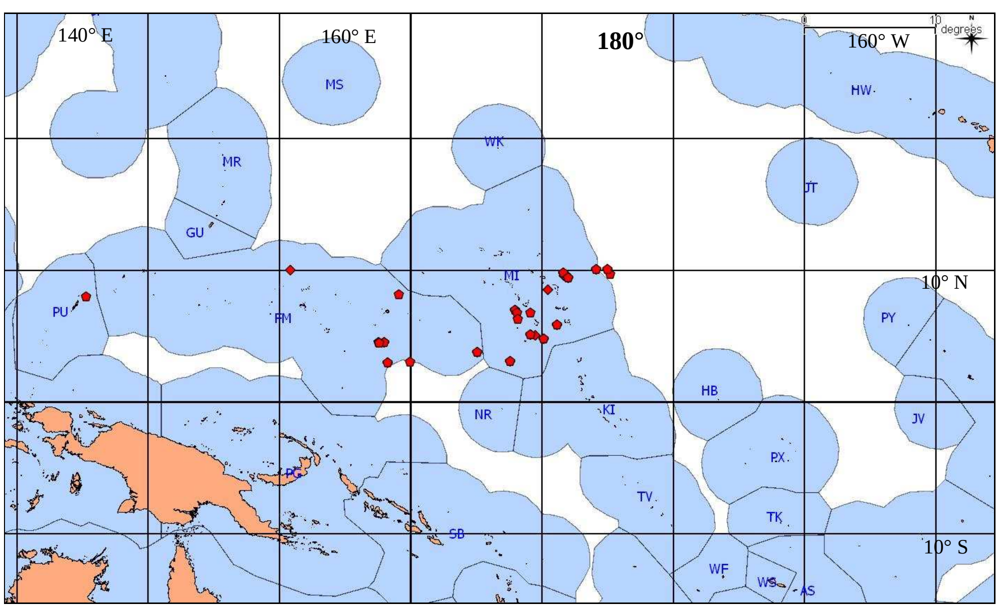

Figure 6. The spatial distribution of otoliths samples collected in FSM, RMI and Palau.

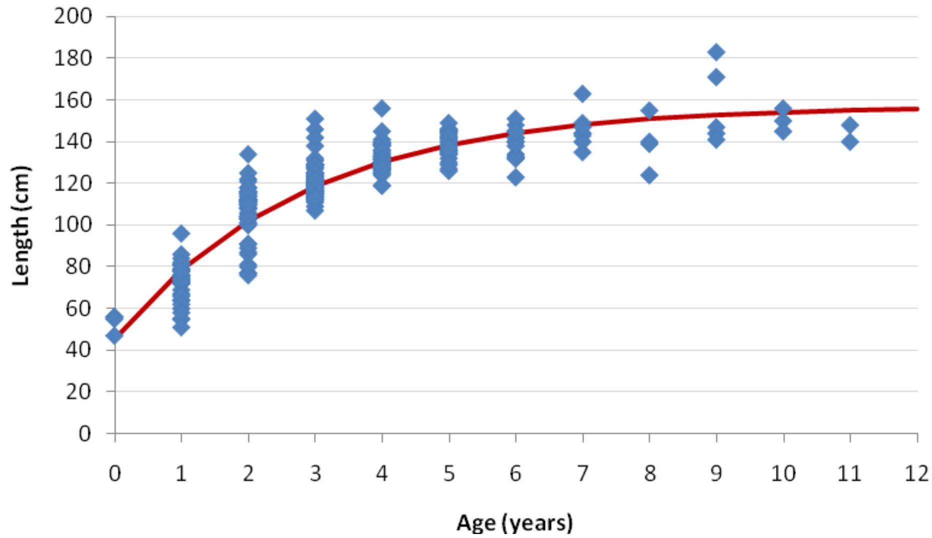

Figure 7. The length at age relationship for the 289 individuals aged by counting annual increments on section otoliths from region 3 of the bigeye stock assessment. The red line is the estimated von Bertalanffy growth function.

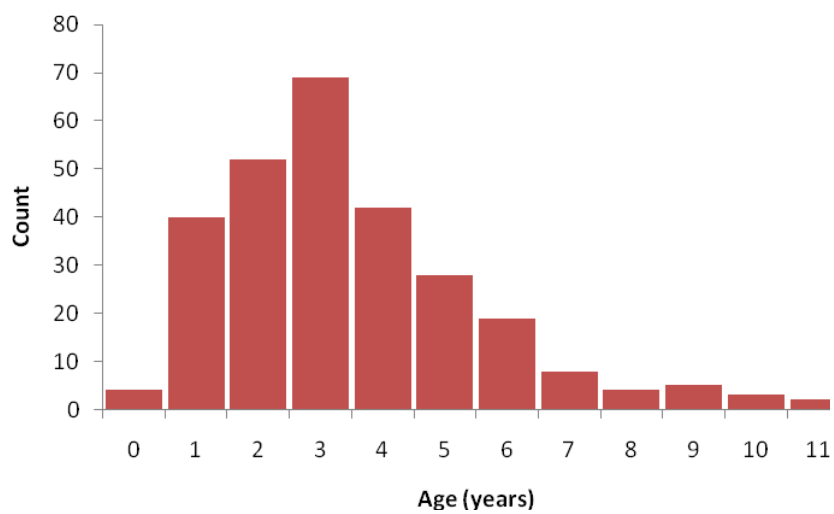

Figure 8. The age frequency distribution for the 289 individuals aged by counting annual increments on section otoliths from region 3 of the bigeye stock assessment.

Table 7. The von Bertalanffy growth parameters for all growth curves estimated in the Pilot study. Note *k* is estimated in qtr^{-1}.*length at age 0.

	Length (Age 0.25)	L_∞	k
Region 1 (MFCL)	25	191.5	0.0622
Region 2 (MFCL)	25	206.5	0.0468
Region 3 (MFCL)	25	157.3	0.0943
Region 3 Otoliths	**23.9***	**149.2**	**0.1098**
Region 4 (MFCL)	25	178.8	0.0700
Region 5 (MFCL)	25	165.6	0.0821
Region 6 (MFCL)	25	165.6	0.0787
Global (MFCL)	25	179.3	0.0698
Region 5 Otoliths	72.4*	166.7	0.0609

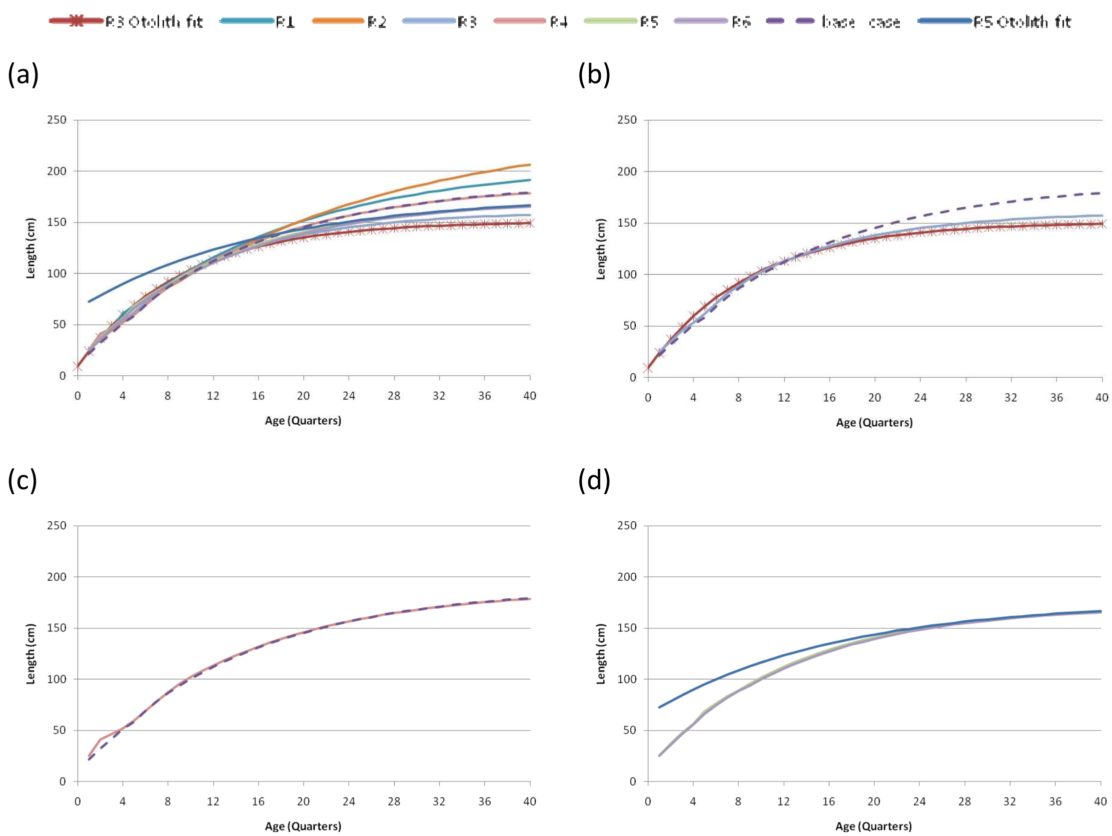

Figure 9. The growth curves for bigeye tuna. (a) all growth curves considered (MFCL for regions 1-6, MFCL global estimate, estimates for region 3 and for region 6 otoliths); (b) growth curve for region 3 from MFCL and otoliths and the MFCL global estimate; (c) Region 4 MFCL estimate and global estimate; (d) Region 5 MFCL estimate and region 5 otloith estimate.

To examine the sampling requirements for the full Pacific-wide study we tested, using change in BIC, latitudinal differences in growth by comparing a combined model (growth model derived using otolith data from region 3 and 5) versus models that estimated the growth for each region separately. For this test, region 5 was subdivided into North Queensland and South Queensland sub regions. As there were no samples below 70cm from region 5, we also excluded samples that were < 70cm from region 3. There was statistical support for the inclusion of spatial differences in growth with the combined BIC of the individual models significantly higher than the combined model (

Table 8), suggesting that minimal sample sizes of 300 are adequate to detect latitudinal differences in growth. To test for longitudinal differences in growth we used a subset of the region 3 otolith data. For this test the combined model included data from the EEZs of Palau, Federated States of Micronesia and the Marshall Islands (combined sample size of 264; 51, 127, and 86 for Palau, FSM, and Marshall Islands respectively). There was no statistical support for the inclusion of spatial differences in growth (

Table 8) indicating that sample sizes of approximately 100 will be insufficient to detect longitudinal differences in growth between regions.

Table 8. BIC statistics for the test of latitudinal and longitudinal differences in growth

Latitude		Longitude	
Model	BIC	Model	BIC
Combined	12460.37	Combined	1984.944
North R5 Queensland	4369.449	Palau	357.5908
South R5 Queensland	5804.797	FSM	989.8999
R3	1984.944	Marshall Islands	648.361
delta BIC	301.1821		-10.908

<u>Comparison of section otolith ageing methods</u>

In addition to the above, 26 individuals were selected where both otoliths had been sampled for a comparison of ageing methods. These fish were sampled in Area 3 (n=12) and Area 4 (n=14). One otolith was prepared for daily increment reading from a longitudinal section following the methods of Schaefer and Fuller (2006). Two transverse sections were cut from the other otolith and 1 prepared for daily reading and the other for annual reading. There was good correspondence between the two daily methods until 21 months (Figure 10). After 21 months there was a higher occurrence of discrepancies between age estimates (Figure 10). The agreement between the age estimates from daily ageing of longitudinal sectioned otoliths and annual increment ageing of transverse sectioned otoliths was high with disagreement only observed once (age 0 for the daily ageing, and age 1 for annual ageing, Figure 11). The agreement between the age estimates from daily ageing of transverse sectioned otoliths and annual increment ageing of transverse section otoliths was less precise (Figure 11). Two individuals were aged 0 by the daily method when the annual estimate was age 1, one individual was aged 2 by the daily method and 3 by the annual method and one individual was aged 4 by the daily method and 5 by the annual method (Figure 11).

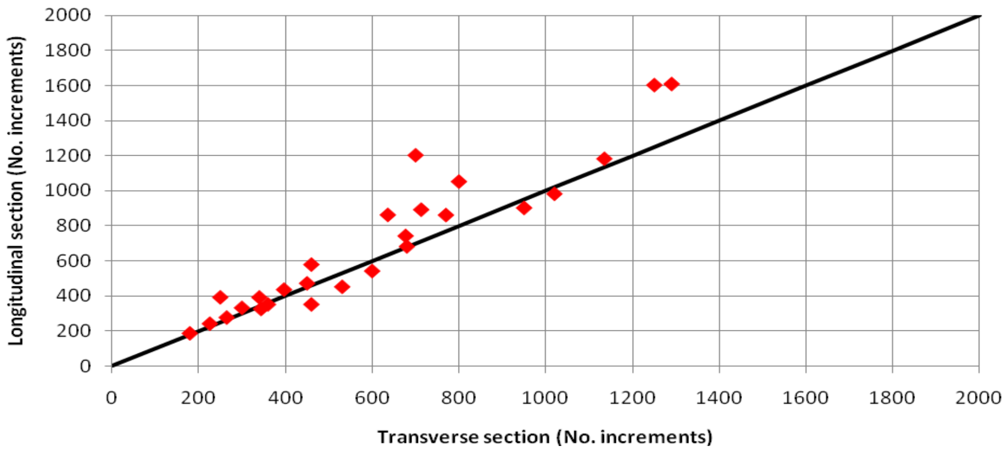

Figure 10. Comparison of estimates of age using daily increment counts from transverse and longitudinal sections from otoliths from the same individual.

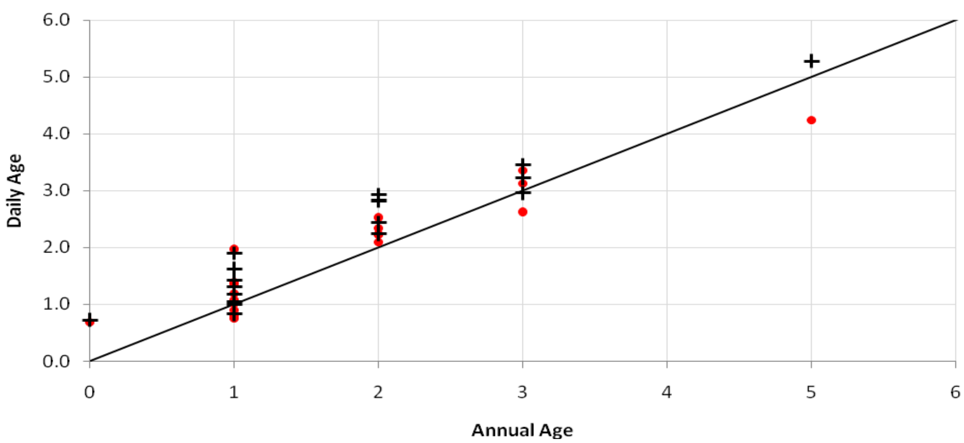

Figure 11. Comparison of estimates of age using daily increment counts from transverse and longitudinal sections and annual increments from transverse sections from otoliths from the same individual. Orange circles represent the correspondence between daily transverse section estimates and annual transverse estimates and crosses represent the correspondence between daily longitudinal section estimates and annual transverse estimates. For the daily estimates age is decimal age.

6.0 Stock Assessment Sensitivity

Correspondence between the age frequency

To compare the estimates of the age composition of the longline fishery in region 3, the frequency of the estimated age of the 2008 and 2009 catch for the LL TW-CH 3 Chinese-Taipei Offshore China, FSM, Marshall Islands, Philippines and Indonesia longline fishery (Fishery 5) for region 3 was extracted from MFCL 2011 bigeye assessment model and the average of this data was compared to the age frequency the otoliths data for region 3 for the same fishery. The age composition for 2010 could not be extracted from the assessment model as the size/weight data for this fishery has not yet been provided to SPC for inclusion. There was a higher frequency of fish aged 2 in the otoliths data and a higher proportion of fish aged 4 in the MFCL estimate (Figure 12). This result is consistent with the differences in the growth curve for region 3 and the base case of MFCL. The potential for non-random sampling may also explain the results.

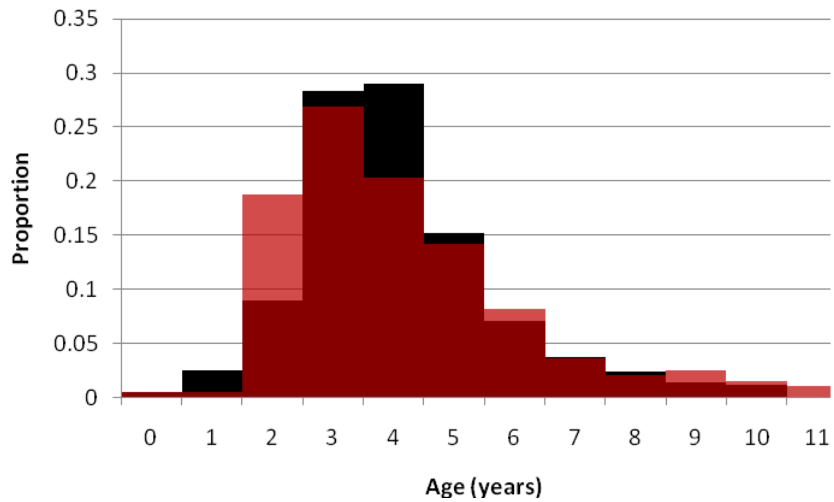

Figure 12. Age frequency distribution for the region 3 otolith data (red bars) and the average for 2008-2009 for Fishery 5 (LL TW-CH 3 Chinese-Taipei Offshore China, FSM, Marshall Islands, Philippines and Indonesia longline fishery) as estimated by MFCL for the 2011 bigeye stock assessment.

<u>Stock Assessment sensitivity to region 3 maturity ogive and growth curve.</u>
The results of SC-6 requested sensitivity analyses of the influence of using the maturity ogive and growth curve estimated by the pilot study are presented in Table 9.

Table 9. Influence of the pilot study maturity ogive and growth curve on the F/Fmsy and SB/SBmsy reference points relative to the reference case for the 2011 bigeye stock assessment.

	$SB_{current}/SB_{msy}$	Relative to base case	$F_{current}/F_{msy}$	Relative to base case
Reference case	1.187	1	1.46	1
Maturity Ogive	1.235	1.04	1.49	1.02
Growth Curve	1.642	1.38	1.09	0.74
Maturity + Growth	1.670	1.41	1.02	0.70

7.0 Recommendations for the Pacific wide study

The results from the pilot study provide some clear guidance for the WCPFC decisions on implementation of the full Pacific-wide study. These being:

1. The maturity L_{50} estimated for the pilot study was 105.9 cm which was consistent with other estimates for the WCPO from equatorial waters (105 cm (Sun et al.

2006); sampling area = 7°-20°N Latitude, 110°-131°E Longitude (region 3); and 107.8 cm (Zhu et al 2010); sampling area = 8°S-6°N Latitude, 153°-134°W Longitude (Region 4)). Farley et al. (2006) estimated a slightly smaller L_{50} of 102.4 cm for Region 5 of the bigeye stock assessment (sampling area = 35°-12°S Latitude, 142°-160°E Longitude) suggesting some larger variation in the maturity ogive in more temperate latitudes or in association with the different depth that the fish were sampled between the studies. The estimate of L_{50} for the EPO is 135 cm (Schaefer et al. 2005). Based on the assumption that there are no significant sampling biases between the various studies undertaken in the Pacific Ocean, these observations suggest that significant changes in the maturation dynamics of bigeye tuna occur east of 130°W longitude. Understanding the mechanisms that cause this change are likely to have important consequences for the model structure of any future Pacific-wide bigeye tuna stock assessment. The continued sampling of ovaries for females >80cm would be required to understand such mechanisms.

The maturity ogive from the EPO has been used in the recent stock assessments for bigeye in the WCPO. The spawning biomass reference point in the 2011 bigeye stock assessment was marginally improved when the pilot study maturity ogive was used. There was little influence of the maturity ogive on the estimate of the fishing mortality reference point. This level of sensitivity was also noted in the analyses performed on the 2008 bigeye stock assessment (Hoyle and Nicol 2008). The pilot study maturity ogive better describes reflect the biology of bigeye in the WCPO and it is recommended that future assessments use a maturity ogive that incorporates the data from the WCPO.

The collection, storage and transport of gonads create logistical difficulties that result in significant financial costs. Options to fix samples in port with formalin should be explored to minimise transportation costs. Given the low level of variation observed in the maturity ogive for the WCPO, it is recommended that further refining the spatial variation in this parameter is a lower priority for the WCPO region of the full Pacific-wide study. A more targeted study to understand the mechanisms varying the reproductive biology between the WCP and the EPO is warranted.

2. The correspondence between the growth curve estimated from the pilot study and that generated from MFCL for region 3 provides preliminary information that the length based information of MFCL is consistent with that derived from otoliths.

   Evidence of spatial variation in growth in both latitude and longitude within the WCPO was confirmed through comparing the parameter estimates for each of the 2011 bigeye stock assessment regions estimated by MFCL and by formally testing for spatial variability in growth between the otoliths data collected during the pilot study and that collected in the Coral Sea in Region 5 of the 2011 bigeye assessment (Farley et al. 2006). In addition, the magnitude of difference between the estimate of growth from the pilot study and the estimate used in the 2011 assessment base case significantly influenced the estimate of the fishing mortality reference point with the fishing mortality estimate more optimistic when the pilot study growth data was used.

   The spatial variation in growth observed has important implications for future structuring of the WCPO bigeye stock assessment. The fishing mortality reference point estimated by MFCL would be more accurate and precise if variation in growth was modeled explicitly. The present construct of MFCL does not allow for spatial differences in growth and substantial modifications would need to be implemented to the code of MFCL to allow this capacity. The development of a MFCL simulator would be beneficial to guide code modifications. A more comprehensive understanding of the biological processes that result in spatial variation in growth would also significantly benefit future model structures for bigeye and also guide the modification to code. The implementation of the Pacific-wide study on growth would provide primary data for developing this understanding.

The research plan suggested a coarse spatial stratification of 32° longitude X 20° latitude for the Pacific-wide study. The results from the pilot study suggest that sample sizes of 300 otoliths are adequate to detect latitudinal differences in growth. Sample sizes of 100 were insufficient to detect longitudinal differences in growth. Consequently it is recommended that 300 otoliths per strata is the minimum sample size required. This is consistent with that recommended in the research plan for the coarse resolution design. Differences in growth by sex have recently been observed in albacore tuna in the WCPO (see Farley et al. 2011) and it is likely that differences by sex also occur in bigeye. It is advised that during the implementation of the Pacific-wide study, a review of the adequacy of the 300 samples per strata is undertaken to ensure that sampling is sufficient to test for sex differences with adequate precision.

There is considerably less cost associated with annual ageing of otoliths in comparison to daily ageing. Given the close correspondence between all otolith-based methods it is recommended that annual ageing be adopted for the full Pacific-wide study.

Estimates of the proportion at age in the catch for each bigeye fishery would also enhance the accuracy and precision of the stock assessment model. The collection of 300 samples per strata for the estimation of growth would be sufficient to develop reliable age-length/weight keys. These keys can be used to age the catch on the basis that adequate length/weight measurements are made for the catch for each fishery. An alternative would be to undertake additional otolith sampling following a random sample design. Determination of the minimum number of random samples would need to be determined by an additional study.

3. The training of PIRFO observers will need to continue if they are to assist with the collection of samples for the Pacific wide study. It is recommended that the Scientific Committee and Commission provide guidance to PIRFO programs on the priorities for biological sampling over the short to medium term (ie. 1-5 years) so that training and logistical support for samples can be included in PIRFO plans and budgets.

   The work requirements for at sea observers are now extensive and biological sampling is typically feasible for only 1 in every 3 sets. If the level of observer coverage on longline vessel at the time of implementation is low (<5%) then strong consideration should be given to dedicated trips for collection of biological samples to ensure that the spatial and temporal coverage of samples is adequate.

   For the fisheries that are landing fresh tuna, preference from fishers will be for otoliths extraction in port. In these cases, the cable tagging approach (or similar) should be adopted to ensure that sex/gonad is matched with the individual.

Training of port samplers for otolith extraction in these ports will be a requirement. It is likely that financial assistance will be required for developing states and participating territories to implement this aspect of the project.

4. The most cost effective method for implementation of the Pacific-wide study would utlise all existing regional and national observer programs operating in the WCPO. To realize this opportunity it is recommended that WCPFC members and regional bodies make a commitment to implementation of the Pacific-wide study and a coordinator in each of these programs is identified to collaborate with the WCPFC secretariat. The successful implementation of the Pacific-wide study will also require a level of central coordination to ensure adequate sampling of each strata. It is recommended that the WCPFC secretariat undertake this role (duties of existing staff or through its science provider).
5. A central repository to store and archive samples is required to ensure access to the samples and data for all WCPFC members. CSIRO has provided this service to the pilot project, however some financial support would be required if it was to provide a similar service to the Pacific-wide study.
6. The budget for implementing the WCPO component of the Pacific wide study is USD90,000 per year over 3 years. This budget is based on USD50,000 for sampling support to developing states and participating territories and project coordination by WCPFC Secretariat, USD15,000 for travel & freight, USD 5,000 for sample curation and USD20,000 for analyses of samples. All other costs (eg observer participation) are assumed to be contributions from member countries.

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