Chair's Report of the CCSBT Maturity Workshop

Bali, Indonesia, 7 - 8 May 2019

1. Opening, adoption of the agenda and meeting arrangements

The CCSBT Maturity workshop was held from 7-8 May 2019 at the Research Institute of Tuna Fisheries (RITF) in Bali, Indonesia. Mr. Zulkarnaen Fahmi, Head of RITF, opened the meeting and welcomed the participants and wished them a successful and productive meeting. The Workshop Chair, Ms. Jessica Farley (CSIRO) also thanked the participants for travelling to the workshop and for collecting and analysing SBT ovaries to include in the analysis. She presented the draft agenda and noted that it focussed on key areas of reproductive biology and maturity relevant to southern bluefin, but it was important for the Agenda to be flexible regarding the practical sessions and discussions. The Agenda was adopted without change and is attached as Appendix A. The list of participants is given in Appendix B.

2. Goals of the workshop

The Chair noted that the goals of the workshop were to:

1) Review ovary sampling programs and identify if (spatial) gaps exist;
2) Review methods to classify female reproductive status and maturity from histological sections of ovaries;
3) Ensure consistent methods were used to classify reproductive state/maturity status;
4) Agree on a histological classification system;
5) Discuss the preparation of a histology-based classification manual for SBT;
6) Develop a preliminary maturity ogive, if time permitted.

3. Overview of SRP project proposal

Ms. Farley presented an overview of the CCSBT Scientific Research Program (SRP) project proposal for estimating size/age at maturity of SBT. She provided background information on previous studies that estimated a maturity ogive for SBT, from the early work of Warashina and Hisada (1970) and Shingu (1970) to the more recent work by Davis et al (2001). Recent estimates of the length at which 50% of females are mature have been around 150-160 cm fork length (FL), however some estimates are outside this range, and most of these estimates were not based on direct observations of ovaries. Thus, it has been recognised that the maturity schedule for SBT is not well understood and that there is a need to develop an improved maturity ogive with which to estimate effective reproductive output, and to inform the conditioning of operating models for stock assessment and MP testing. It is also important that an agreed and appropriate histological classification scheme is used to differentiate mature from immature females.

Ms. Farley explained why it is complicated to estimate an unbiased maturity ogive for a species, such as SBT, which migrates from various feeding grounds to a separate spawning area to spawn. Bias towards mature or immature fish can occur depending on the time and area from which fish are sampled. The objectives of the SRP Project were presented:

i. Collect ovary and otolith samples from females ≥ 110 cm FL from the main fisheries in southern oceans in Apr-Aug (austral winter).
ii. Determine if immature females can be distinguished from regenerating females from histological sections of ovaries using maturity markers.
iii. Develop a standardised ovary histology classification scheme.
iv. Develop appropriate statistical models to estimate a maturity ogive for females.
v. Conduct a CCSBT maturity workshop to review the histological methods and collate on results.
vi. Develop a manual with classification scheme for future use.

It is important that the timing of sampling targeted areas and times when immature and mature fish are mixed on their feeding grounds, soon after spawning is complete. At that time of year (Apr-Aug), however, regenerating and developing (repeat spawning) females can be found and may be mistaken as immature and developing (first time spawning). Microscopic (histology) assessment and calibration is essential to correctly stage each fish.

4. Proposed criteria to classify maturity status in SBT

Ms. Farley briefly reviewed ovary organisation, development and classification. Fish ovaries are classified according to the pattern of the egg (oocyte) development. SBT have "asynchronous" development because oocytes at different maturation stages are found in their ovaries at the same time. SBT are also batch spawners. The standard terminology/system for describing reproductive development, and the reproductive cycle for batch spawners, was provided. The proposed criteria to differentiate mature from immature females (ovaries) caught after the end of the spawning season was reviewed. This can be done using oocyte stage, oocyte atresia and "maturity markers" present in ovary histology. Attention was largely focused on the maturity markers, which indicate prior spawning activity. The markers included "brown bodies", muscle bundles, residual hydrated oocytes, and the presence of a thick ovarian wall. "Brown bodies" are melano-macrophage centres associated with 'chronic inflammatory lesions' and develop in association with ovary atresia. Muscle bundles are connective tissue bundles that become evident after spawning has ceased and the ovary has contracted/collapsed. At this time, the oocytes in the ovary become disorganised with more space, interstitial tissue and capillaries around primary growth oocytes, and the ovary wall appears thicker than is the case for an immature fish. Hydrated oocytes may be embedded in the ovary lamella or lumen. The criteria described can be used to identify immature and developing (first time) females from regenerating and developing (repeat spawning) females. The proposed classification scheme is shown in Appendix C).

A question was raised about why females are predominantly studied. In most species, females are the limiting sex, in terms of reproductive output, as a single male can fertilize the eggs of many females. It is also much more difficult to estimate reproductive parameters in males, so more attention has been focused on females.

5. Proposed statistical methods to estimate the maturity ogive

Ms. Paige Eveson presented statistical methods for estimating maturity ogives. Specifically, she presented a method for fitting a logistic model to the proportion of mature females by length (or age) using a generalized linear model with a binomial distribution and a logit link function. This can be done in the statistical software R using the function 'glm'. The model was fit to simulated data sets to illustrate the outcomes if maturity ogives differ spatially. If maturity ogives differ spatially

and a single model is fit to all data pooled across areas, then the estimated maturity ogive will be biased (see Appendix D). In order to get an unbiased ogive, it is necessary to have estimates of the relative proportion female abundance in each area by length class in the population compared to that in the sample. Then a single logistic model can be fit to all of the data, but with the data weighted according to the relative distribution of samples and abundance among areas. For example, consider 2 areas: if for a given length class *l*, the population proportion of females is 75% in area 1 and 25% in area 2, whereas the sample proportions are 50% in area 1 and 50% in area 2, the weight to be given to the data in length class *l* from area 1 is 0.75/0.50=1.5 and from area 2 is 0.25/0.50=0.5. These weights can easily be incorporated in R using the 'weights' argument for the 'glm' function (see Appendix D).

6. Presentations on ovary sampling and histology/maturity staging used by Institutes

SBT ovaries were collected by Australia, New Zealand, the Fishing Entity of Taiwan and Korea. A summary of the sampling program and processing (i.e., maturity status determined using histological methods) was presented.

Australia and New Zealand

Ms. Farley provided an overview of the ovaries sampled and analysed by Australia and New Zealand. A total of 58 ovaries were collected from CCSBT statistical area 5, 109 from area 6 and 198 from area 7. Fish were sampled from May to August, with the majority sampled in June. The size range of fish sampled differed slightly among areas with slightly smaller fish sampled in area 7 and slightly larger fish sampled in area 5 (see Figure 1 1). Using the proposed histological criteria to classify reproductive development and maturity status, five reproductive phases were observed in the samples: immature, early developing (immature), later developing (immature), regenerating (mature) and early developing (mature). Based on this data, preliminary maturity ogives were presented for each area.

The Fishing Entity of Taiwan

Dr. Ching-Ping Lu presented an overview of the ovaries sampled and analysed by the Fishing Entity of Taiwan. A total of 569 ovaries were collected by scientific observers from fish caught between April and September in the years 2010 to 2017 in the region straddling statistical areas 2 and 8 (labelled as Area 8 in Figures below). Females ranged in size from 80 to 178 cm FL, although the majority were between 100 and 140 cm FL (see Figure 1). Sexual maturity was determined based on histological sections of the gonad samples. Since the criteria of gonadal developmental stages were not available for SBT, the criteria of Farley et al. (2014) for albacore in the southern Pacific Ocean were adopted to categorize the gonadal developmental stages for SBT. Preparations of histological sections failed for some samples due to frozen preservation process. The majority of females were classified as immature, developing or regenerating. The proportion of fish by histological classification were presented by length class.

Korea

Dr. Sung Il Lee presented an overview of the ovaries sampled and analysed by Korea. A total of 365 ovaries were collected by scientific observers from fish caught between April and September in years 2015-2017 in statistical areas 8 and 9. The majority of fish samples were 100 to 150 cm FL. Histological sections have only been prepared for fish caught in 2015. Monthly changes in maturity stages and in the gonadosomatic index between April and September were presented.

7. Practical sessions (microscope work)

A primary component of the workshop was a series of practical sessions viewing ovary histology using microscopes and an image analysis system. This permitted material to be viewed by all participants simultaneously. The practical work consisted of a combination of:

- Group sessions that reviewed histological slides provided by participants of ovaries at different development and maturity stages.
- Individual microscope work to review and discuss histological criteria and classification.
- "Testing" of skills gained in histology classification by participants from the workshop.

During the group sessions, important structures in SBT ovaries were identified including oocytes at different stages of development, atresia at different levels of resorption (alpha and beta), and different types of maturity markers present in SBT ovaries that can be used to identify mature-regenerating females. Ovaries considered "easy" to interpret and identify key features were viewed first. These included ovaries from very small and very large fish to clearly show the similarities and differences in the ovary sections of immature and mature-regenerating ovaries. A number of "difficult" ovaries were then viewed from middle-sized fish, which could either be immature or mature based on fish size. It is critical that the ovaries of these fish are staged correctly, and a substantial amount of time was devoted to these samples. Both fresh-fixed and frozen-fixed samples were examined to demonstrate differences in the histology preparations.

Many participants were unfamiliar with histological analysis of gonads. Time was spent viewing ovary histology with participants individually and reviewing histological stages assigned prior to the workshop. As expected, the classifications were often correct for the smallest and largest fish, but were mixed for the more difficult middle-sized fish. Towards the end of the practical sessions, a short 'calibration' exercise was undertaken where participants (in groups of 2-3) were provided with three histological sections to score independently. The overall results of this exercise were good, suggesting that participants were able to successfully identify "maturity markers" and classify fish into reproductive phases (mature or immature) by the end of the workshop.

The participants agreed that the proposed criteria to classify maturity status in SBT was suitable, but they identified the need for an illustrated histological identification guide to be able to complete the histological staging of their respective samples. The manual was to include common terminology, document key structures used to stage females, and the classification scheme proposed for SBT. The manual could then be used by scientists in each member country to correctly stage each female sampled. The Chair agreed to develop the manual after the workshop and also to finalise a "key to tuna reproductive phases based on ovary histology".

The workshop also highlighted the need for good quality histological sections, from fresh-fixed ovary material, if possible. Ideally, the histological sections would be prepared from a sample of a full cross-section of the ovary so that the both the ovary wall and lumen were visible. This provides sufficient material for the reproductive status to be reliably assessed. This would ensure consistent methods were used to classify reproductive state and maturity status.

Although additional training may not be required to complete the analysis, cross-validation of ovary histology is recommended to ensure consistency. It may be possible to complete this using images of histological features of suitably prepared ovaries. The Chair agreed to continue to help participants to correctly identify structures in the histological sections subsequent to the workshop.

8. Review and finalise classification of all ovary samples by members

It was not possible to finalise the classification of all the histology available at the workshop. The illustrated histological identification guide is required before participants can re-evaluate their ovary histology. Additional samples are also being collected by some countries for inclusion in the project.

9. Preliminary data analysis/modelling (maturity ogive estimation)

Although the final set of data were not available for analysis at the workshop, logistic models were fit to the data available for a preliminary investigation of spatial differences between statistical areas. The sample sizes by statistical area are shown in Figure 1. The group noted that the level of sampling was low in most CCSBT statistical areas except areas 7 and 8 (the aim was to sample ~220 females per area).

The estimated maturity ogives by area are shown separately in Figure 2 (mean and approximate 95% confidence bounds), and for ease of comparison, the mean curves are plotted together in Figure 3. The ogives for areas 5 and 7 are very similar; the ogive for area 4 appears to be slightly less steep, however the sample size for this area is small and the estimated proportions mature do not differ significantly from areas 5 and 7 when confidence intervals are taken into account. On the other hand, the maturity ogive for area 8 is significantly different from areas 5 and 7; note, however, that even though the sample size for area 8 is quite large, most samples are for small, immature fish so the confidence bounds are still wide for the larger length classes (Figure 2). The data for area 8 were provided by the Fishing Entity of Taiwan, and discussions at the workshop suggest that the histological classification (and, thus, maturity status) may be incorrect in some cases. Thus, these samples need to be revisited before drawing any conclusions.

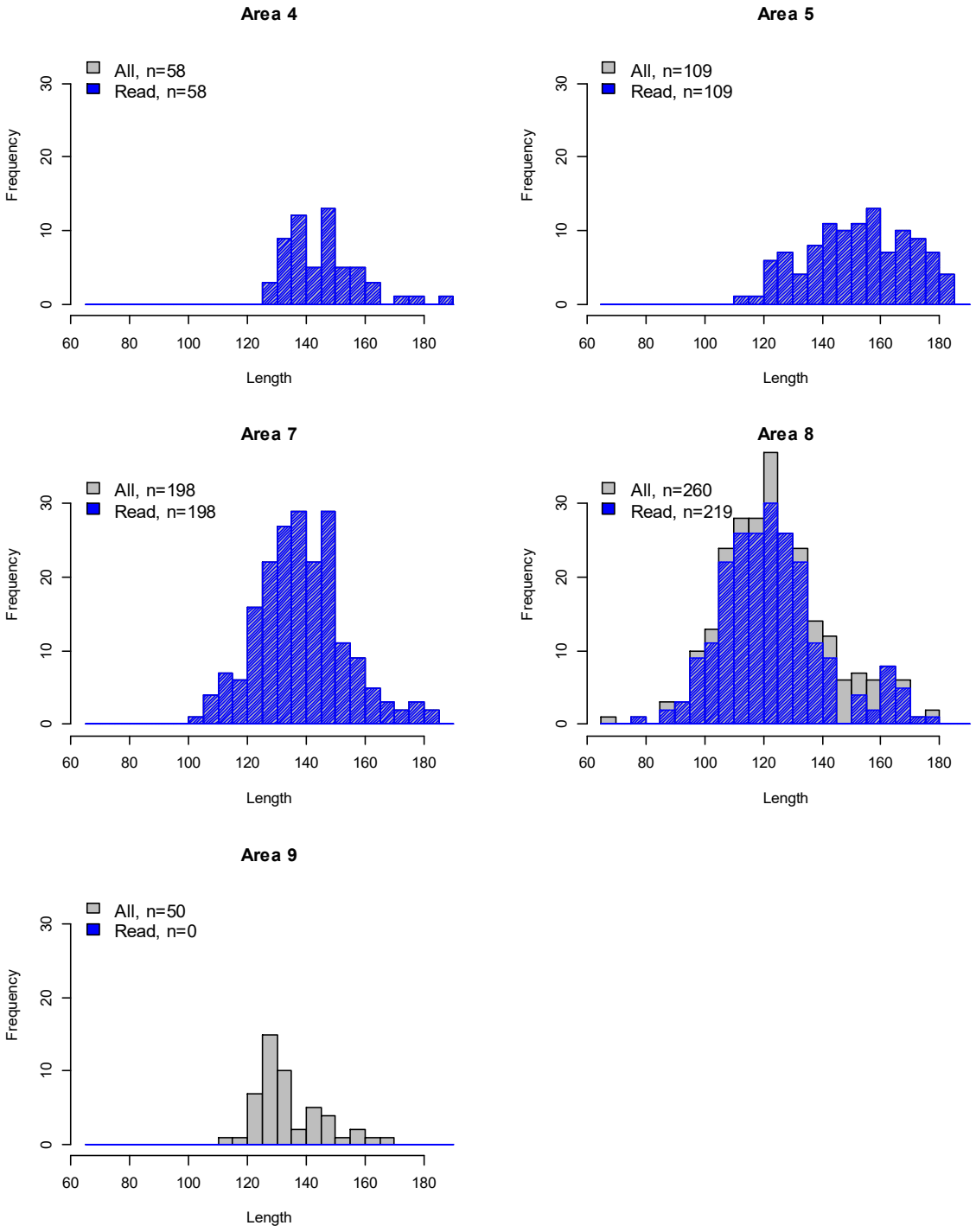


Figure 1. Number of samples (female only) collected and number which have been read (i.e., maturity status determined) by statistical area.

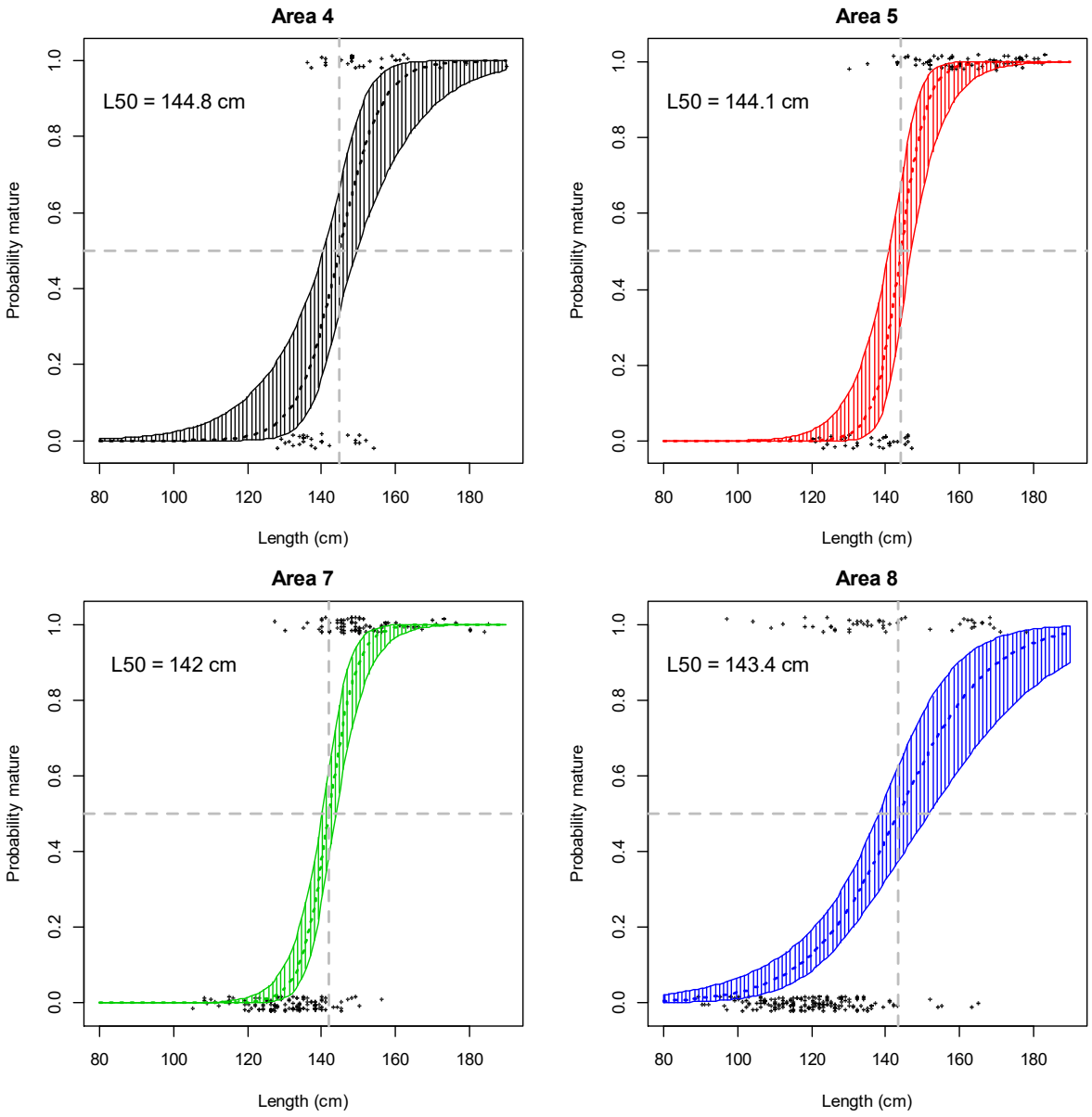


Figure 2. Preliminary maturity ogives fit to the data available at the workshop from each statistical area. The mean proportion mature ±2 standard errors is shown, as well as the estimated length at 50% maturity (L50).

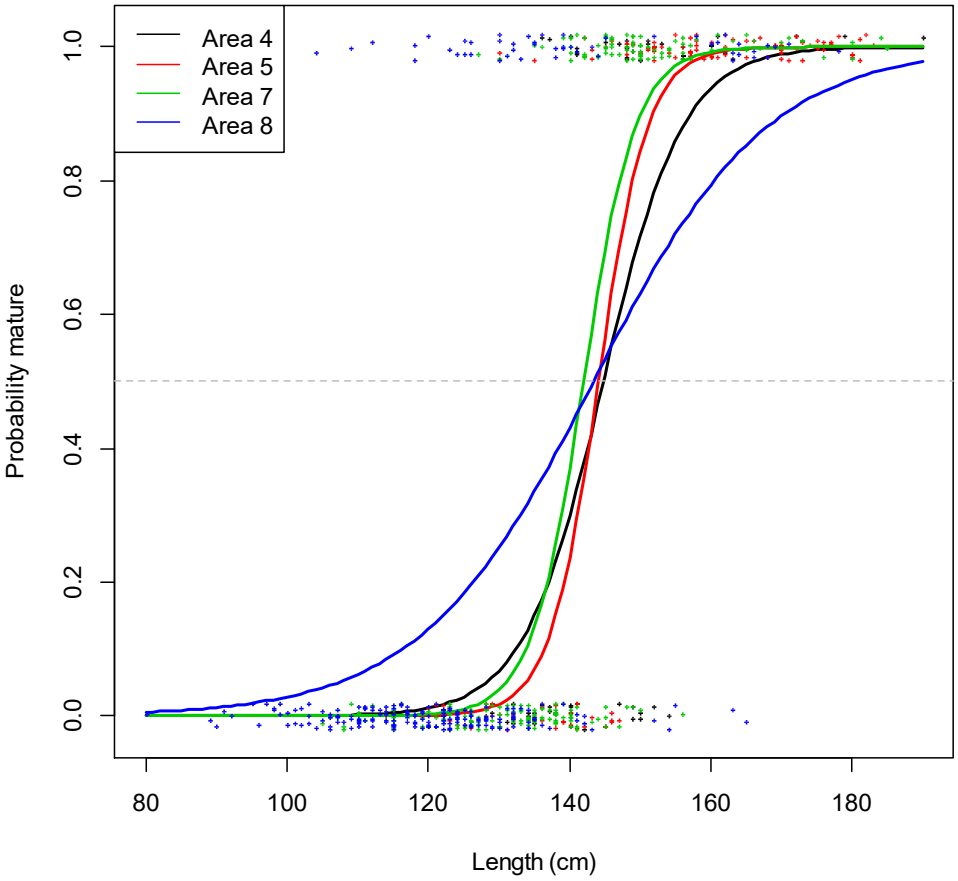


Figure 3. The estimated mean maturity ogives shown in Figure 4.5.2, but overlaid for easier comparison.

10. Initial drafting of manual with the classification scheme and digital images of maturity markers

As noted above, the group agreed that a manual which included the agreed classification scheme for SBT should be developed by the Chair before classifications can be finalised in order to improve consistency.

11. Close of the meeting

The Chair thanked the participants for their contributions and adjourned the meeting.

Appendix A

Agenda

CCSBT maturity workshop

Bali, Indonesia

7-8 May 2019

Tuesday 7 May	Workshop Items	
09:00 – 9:30	Welcome and introduction of participants	Fahmi and Farley
	Administrative arrangements	Farley
	Review of agenda	
	Goals of the workshop	
10:00 – 10:45	Overview of SRP project proposal	Farley
	Proposed criteria to classify maturity status in SBT	
10:45-11:15	*Morning tea/coffee*	
11:15-12:30	Proposed statistical methods to estimate the maturity ogive	Eveson
	Questions and discussion	
12:30 – 13:30	*Lunch*	
13:30 – 17:00	Brief presentations on ovary sampling and histology/maturity staging used by Institutes (including photos of stages)	
	Australia and New Zealand	Farley
	Fishing Entity of Taiwan	Lu
	Korea	Lee
	Questions and discussion on maturity staging	
	Refinement to standardised criteria for classifying each maturity stage	
15:15-15:30	*Short break – afternoon tea/coffee*	
15:30-17:00	Practical session (microscope work)	
	Identify histological features in ovaries (frozen- and fresh-fixed)	
	Classify ovaries into reproductive phases	
	Identify immature and mature females	
	Workshop dinner (venue to be decided)	

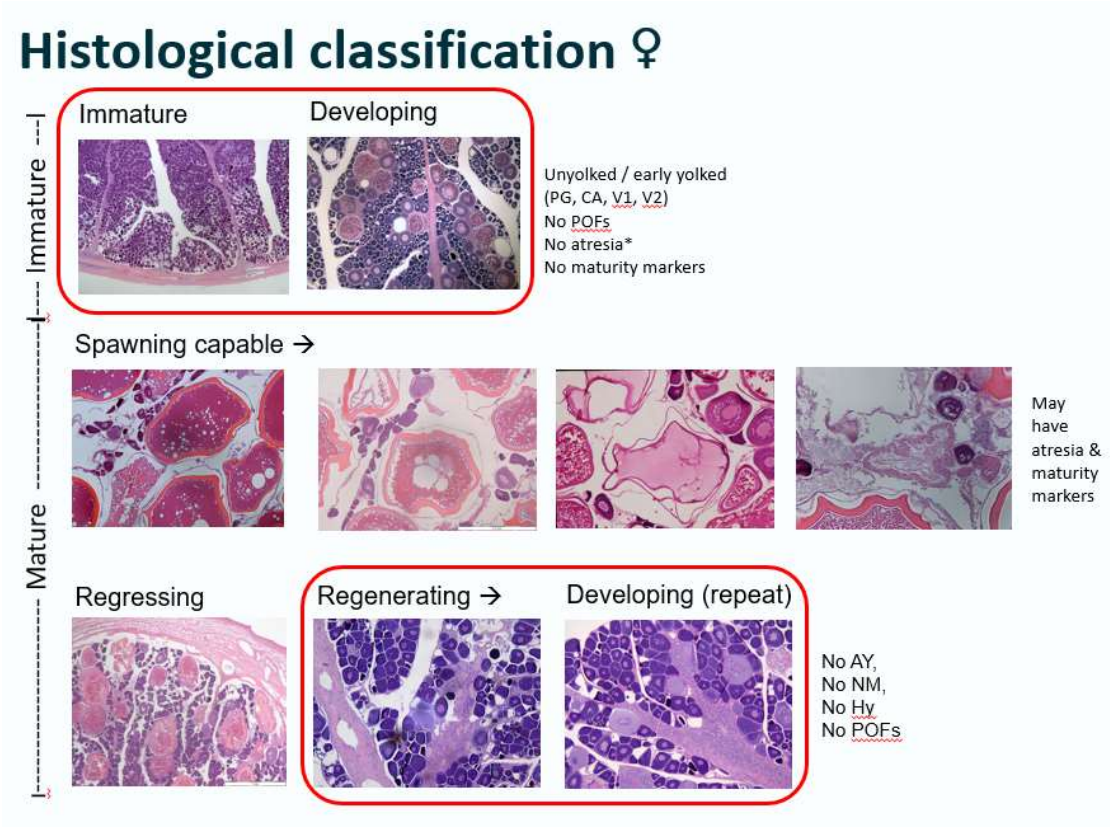
Wednesday 8 May	Workshop Items	
09:00 – 10:45	Practical session (microscope work) continued Calibration exercise - cross reading histology sections Agree and finalise ovary classification scheme	
10:45-11:15	*Morning tea/coffee*	
11:15-12:30	Review and finalise classification of all ovary samples by members Data exploration and data checking	
12:30 – 13:30	*Lunch*	
13:30 – 17:00	Preliminary data analysis/modelling (maturity ogive estimation) if time allows	Eveson
15:15-15:30	*Short break – afternoon tea/coffee*	
15:30-17:00	Initial drafting of manual with the classification scheme and digital images of maturity markers Wrap up discussion	

List of Participants
CCSBT Maturity Workshop

First name	Last name	Title	Position	Organisation	Postal address	Tel	Fax	Email
CHAIR								
Jessica	FARLEY	Ms	Fisheries Scientist	CSIRO Oceans & Atmosphere	GPO Box 1538, Hobart, TAS, Australia 7001	61 3 6232 5189		jessica.farley@csiro.au
MEMBERS								
AUSTRALIA								
Paige	EVESON	Ms	Research Scientist	CSIRO Oceans & Atmosphere	GPO Box 1538, Hobart, TAS, Australia 7001	61 3 6232 5015		paige.eveson@csiro.au
FISHING ENTITY OF TAIWAN								
Ching-Ping	LU	Dr.	Assistant Professor	National Taiwan Ocean University	2 Pei-Ning Road, Keelung 20224, Taiwan	886 2 2462 2192 ext 5035	886 2 2463 3920	michellecplu@gmail.com
INDONESIA								
Zulkarnaen	FAHMI	Mr	Head of Research Institute for Tuna Fisheries	Research Institute for Tuna Fisheries	Jl. Mertasari No. 140, Br Suwung Kangin, Sidakarya, Denpasar, Bali 80224, Indonesia	62 361 726201	62 361 849747	fahmi.p4ksi@gmail.com
Bram	SETYADJI	Mr	Research Coordinator	Research Institute for Tuna Fisheries	Jl. Mertasari No. 140, Br Suwung Kangin, Sidakarya, Denpasar, Bali 80224, Indonesia	62 361 726201	62 361 849747	bram.setyadji@gmail.com
Hety	HARTATY	Mrs	Researcher	Research Institute for Tuna Fisheries	Jl. Mertasari No. 140, Br Suwung Kangin, Sidakarya, Denpasar, Bali 80224, Indonesia	62 361 726201	62 361 849747	hhartaty@gmail.com
Ririk Kartika	SULISTYANINGSIH	Mrs	Researcher	Research Institute for Tuna Fisheries	Jl. Mertasari No. 140, Br Suwung Kangin, Sidakarya, Denpasar, Bali 80224, Indonesia	62 361 726201	62 361 849747	rk.sulistyaningsih.11@gmail.com
Prawira ARP	TAMPUBOLON	Mr	Researcher	Research Institute for Tuna Fisheries	Jl. Mertasari No. 140, Br Suwung Kangin, Sidakarya, Denpasar, Bali 80224, Indonesia	62 361 726201	62 361 849747	parptampubolon@gmail.com
Gussasta Levi	ARNENDA	Mr	Researcher	Research Institute for Tuna Fisheries	Jl. Mertasari No. 140, Br Suwung Kangin, Sidakarya, Denpasar, Bali 80224, Indonesia	62 361 726201	62 361 849747	gussastaarnenda@gmail.com

First name	Last name	Title	Position	Organisation	Postal address	Tel	Fax	Email
Indrastiwi	PRAMULATI	Ms	Lab Analyst	Research Institute for Tuna Fisheries	Jl. Mertasari No. 140, Br Suwung Kangin, Sidakarya, Denpasar, Bali 80224, Indonesia	62 361 726201	62 361 849747	indrastiwipramulati@gmail.com
Desy Shintya	IRENE	Ms	Lab Analyst	Research Institute for Tuna Fisheries	Jl. Mertasari No. 140, Br Suwung Kangin, Sidakarya, Denpasar, Bali 80224, Indonesia	62 361 726201	62 361 849747	irenedesy3112@gmail.com
Ni Komang	SURYATI	Ms		Research Institute for Inland Fisheries and Extension	Jl. Gubernur H.A bastari No. 8 Jakabaring - Palembang, Sumatera Selatan, Indonesia	62 711 564960 00	62 711 564960 1	komang_nks@yahoo.com
Aisyah		Ms		Fisheries Research Center	Jl. Pasir Putih II, Ancol Timur, Jakarta Utara 14430, Indonesia	62 21 647009 28	62 647009 29	icha_saraimanette@yahoo.com
Ria	FAIZAH	Ms		Fisheries Research Center	Jl. Pasir Putih II, Ancol Timur, Jakarta Utara 14430, Indonesia	62 21 647009 28	62 647009 29	faizah.ria@gmail.com
Muhammad	TAUFIK	Mr		Research Institute for Marine Fishery	Jl. Raya Bogor Km. 47 Nanggewer Mekar, Cibinong - Bogor, Jawa Barat, Indonesia			taufik.brpl@gmail.com
Kusno		Mr		Fisheries Research Center	Jl. Pasir Putih II, Ancol Timur, Jakarta Utara 14430, Indonesia	62 21 647009 28	62 647009 29	kusnosusanto@yahoo.com
JAPAN								
Yuichi	TSUDA	Dr	Researcher	National Research institute of Far Seas Fisheries	5-7-1 Orido, Shimizu, Shizuoka 424-8633, Japan	81 54 336 6000	81 543 35 9642	u1tsuda@fra.affrc.go.jp
REPUBLIC OF KOREA								
Doo Nam	KIM	Dr.	Senior researcher	National Institute of Fisheries Science	216, Gijanghaean-ro, Gijang-eup, Gijang-gun, Busan, 46083	82 51 720 2330	82 51 720 2337	doonam@korea.kr
Sung Il	LEE	Dr.	Researcher	National Institute of Fisheries Science	216, Gijanghaean-ro, Gijang-eup, Gijang-gun, Busan, 46083	82 51 720 2331	82 51 720 2337	k.sungillee@gmail.com

Appendix C: Proposed histological classification for SBT.



Maturity status	Phase	Sub-phase	MAGO & POF stage	Atresia of late vitellogenic oocytes	Maturity markers[1]
Immature	Immature		Primary growth or cortical alveoli oocytes, no POFs	Absent	Absent
	Developing (first time)		Early vitellogenic oocytes, no POFs	Absent	Absent
Mature	Spawning capable	Non- spawning	Late vitellogenic oocytes, no POFs	α and β atresia may be present	Possible
	Spawning capable	Actively spawning	Migratory nucleus or hydrated or POF's	α and β atresia may be present	Possible
	Regressing		Primary growth or cortical alveoli oocytes, no POFs	100% in the α or β stages of atresia	Possible
	Regenerating		Primary growth or cortical alveoli oocytes, no POFs	Absent	Present
	Developing (repeat)		Early vitellogenic oocytes, no POFs	Absent	Present

1 = Maturity markers include "brown bodies", muscle bundles, residual hydrated oocytes, disorganised structure, and the presence of a thick ovary wall
POFs = post ovulatory follicles.

Appendix D: Statistical models for estimating a maturity ogive.

Our goal is to estimate a single maturity ogive for the population of female SBT. The most common approach for estimating a maturity ogive is to model the proportion of mature females, p , as a function of either length or age using a logistic model:

$$\log\left(\frac{p}{1-p}\right) = \alpha + \beta x$$

where x is either length in cm or age in years, and α and β are parameters to be estimated. Length is commonly used because age is often not known for many (or all) of the samples, and there is evidence for some species that length is a better determinant of maturity. Using the logistic model, the length (age) at which 50% of females are mature, L_{50} (A_{50}), is given by $-\alpha/\beta$. The model can be fit in the statistical software R using the function 'glm' and specifying the family as binomial and the link function as logit. If the logistic model does not fit will, other link functions are available for comparison (e.g., probit, log, complementary log-log).

For the workshop, simulations were used to illustrate the potential problem of fitting a single maturity ogive to all data if maturity differs spatially. We considered two areas and generated maturity data for a sample of 500 females assuming uniform lengths between 100-200 cm and logistic maturity as a function of length with $\alpha = -23$ and $\beta = 0.15$. If females are distributed randomly between the two areas (Scenario 1), then fitting a single maturity ogive to all of the data gives an unbiased maturity ogive, regardless of how sampling is done (equal or unequal sample sizes by area, random or length-stratified). For example, suppose 100 samples were taken randomly from area 1 and 400 from area 2; then both the area-specific and combined maturity ogives are unbiased (Figure D1), with the difference being that the uncertainty is greater with smaller sample sizes (i.e. uncertainty is greatest for area 1 and smallest for the combined data). Suppose instead that immature females are 75% more likely to go to area 1, and mature females are 75% more likely to go to area 2 (Scenario 2). Then, since maturity status depends on length, the proportion of females in area 1 vs area 2 will differ by length class (Table D1). In this case, if we fit a single maturity model to data pooled across areas, then the estimated maturity ogive will be biased in most situations – the only exception being if samples were taken in proportion to abundance *within each length class*; i.e., in our example, 65% of samples within length class 140-150 cm would need to come from area 1, and 35% from area 2 (Table D1). This will not be the case if sample sizes are unequal between areas and/or sampling is not random (e.g. length-stratified or taken from size-selective catches). Consider the case of random sampling but unequal sample sizes, with 100 samples from area 1 and 400 from area 2. We expect the area-specific maturity ogives to be biased, but the combined ogive is biased as well (Figure D2).

A situation such as Scenario 2 would be true for SBT if we were to sample fish during the spawning season (i.e., the austral summer), since mature fish of a given length or age are much more likely to be found on the spawning grounds than immature fish. For this reason, the SRP proposal specified that samples be collected during the winter months. Nevertheless, there is still the possibility that mature and immature fish are distributed differently in the winter; for example, fish that are mature may be more likely to be found in areas closer to the spawning ground (e.g. southeast Indian Ocean), whereas fish of the same size/age that are immature may be more likely

to be found further away (e.g. the Tasman Sea). By collecting samples from across the geographic range of SBT, we can test for such scenarios.

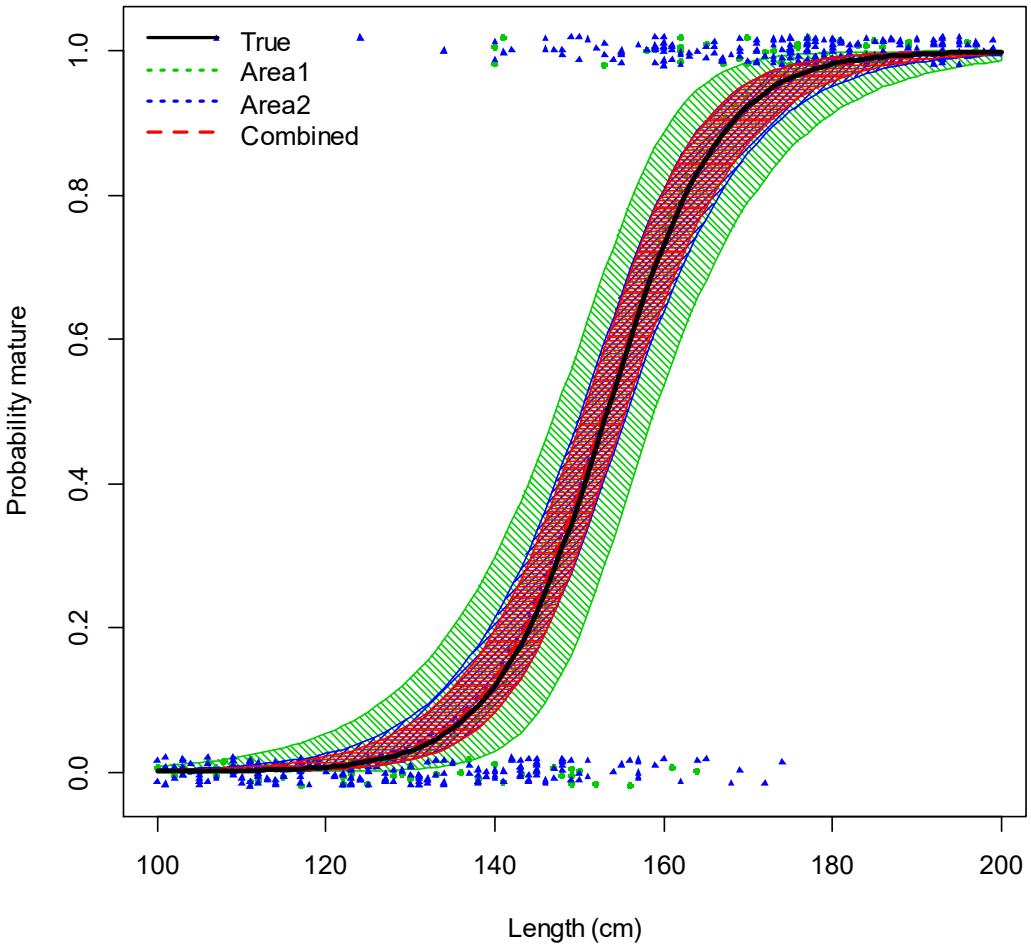


Figure D1. Simulated data and estimated maturity curves for Scenario 1, in which females are randomly distributed amongst 2 areas with 100 samples taken randomly from area 1 and 400 from area 2. The area-specific and combined estimated mean maturity curves (±2 standard errors) are shown. The raw data for each area (0=immature; 1=mature) are also plotted, noting that the points have been jittered to avoid overlap.

Table D1. Proportion of females in each area by length class for simulation Scenario 2.

Length class (cm)	Area 1	Area 2
[100,110)	0.75	0.25
[110,120)	0.76	0.24
[120,130)	0.75	0.25
[130,140)	0.72	0.28
[140,150)	0.65	0.35
[150,160)	0.49	0.51
[160,170)	0.33	0.67
[170,180)	0.27	0.73
[180,190)	0.26	0.74
[190,200)	0.26	0.74

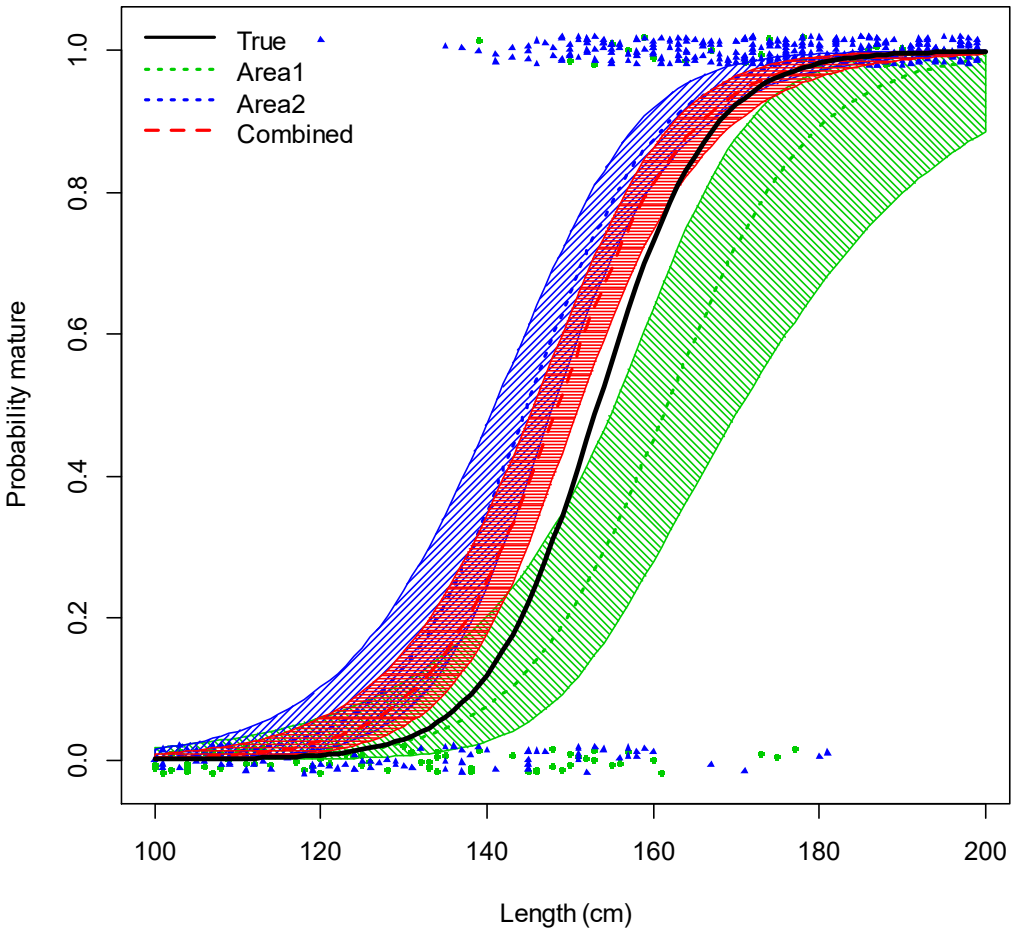


Figure D2. Simulated data and estimated maturity curves for Scenario 2, in which females are 75% more likely to go to area 1 if immature and 75% more likely to go to area 2 if mature. 100 samples were taken randomly from area 1 and 400 from area 2. The area-specific and combined estimated mean maturity curves (±2 standard errors) are shown. The raw data for each area (0=immature; 1=mature) are also plotted, noting that the points have been jittered to avoid overlap.

If in fact maturity ogives do differ by area, it is still possible to estimate an unbiased maturity ogive. To do so requires having estimates of the proportion female abundance in each area by length class in the population compared to in the sample. Then a single logistic model can be fit to all of the data, but with the data weighted according appropriately. For example, in a situation with two areas, if for a given length class *l*, the population proportion of females is 75% in area 1 and 25% in area 2, whereas the sample proportions are 50% in area 1 and 50% in area 2, the weight to be given to the data in length class *l* from area 1 is 0.75/0.50=1.5 and from area 2 is 0.25/0.50=0.5. These weights can easily be incorporated in R using the 'weights' argument for the 'glm' function. In our simulated example for Scenario 2, the proportion of females in each area by length class for the population were given in Table D1, the same information but for the sample is given in Table D2, and the relative weights to be input to the model (population proportions divided by sample proportions) are given in Table D3. We refit a logistic model to the combined data using these weights and the resulting ogive was unbiased (Figure D3). Unfortunately, in most

real situations (such as for SBT), getting reliable data on the numbers of fish by area, length class and sex will be challenging and perhaps not possible.

Table D2. Proportion of females in the sample in each area by length class for simulation Scenario 2, when 100 random samples are taken from area 1 and 400 from area 2.

Length class (cm)	Area 1	Area 2
[100,110)	0.36	0.64
[110,120)	0.41	0.59
[120,130)	0.36	0.64
[130,140)	0.56	0.44
[140,150)	0.23	0.77
[150,160)	0.11	0.89
[160,170)	0.13	0.87
[170,180)	0.11	0.89
[180,190)	0.07	0.93
[190,200)	0.05	0.95

Table D3. Relative weights to be given to the maturity data in each area by length class for simulation Scenario 2, when 100 random samples are taken from area 1 and 400 from area 2. These weights are calculated by dividing the proportions for the population in Table D1 by those for the sample in Table D2.

Length class (cm)	Area 1	Area 2
[100,110)	2.10	0.39
[110,120)	1.84	0.41
[120,130)	2.09	0.40
[130,140)	1.30	0.62
[140,150)	2.84	0.46
[150,160)	4.25	0.58
[160,170)	2.52	0.77
[170,180)	2.49	0.82
[180,190)	3.62	0.80
[190,200)	5.11	0.78

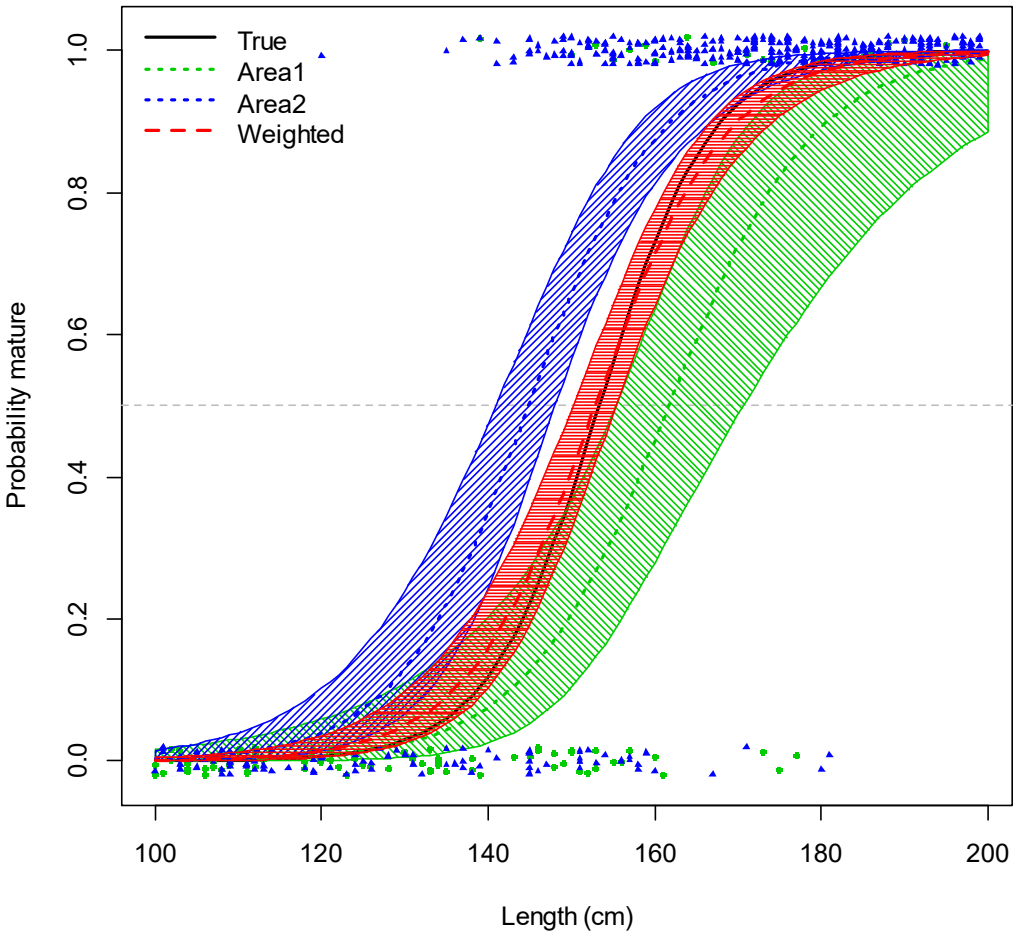


Figure D3. Same as Figure D22 except in this case the maturity ogive for the combined data was obtained by fitting a logistic model to weighted data using the relative weights in Table D3.