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Update on the length and age distribution of SBT in the Indonesian longline catch and close-kin tissue sampling and processing.

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Contents

1 Abstract

In 2013, the Extended Scientific Committee (ESC) developed a new Scientific Research Plan for southern bluefin tuna (SBT). The SRP was reviewed in 2014 and again in 2015. Several items were identified as high priority in the 2016 work plan including the ageing of Indonesian SBT otoliths and continued collection and genotyping of tissue samples for close-kin genetics. In this paper we provide an update on progress in these activities.

In 2015/16, otoliths and muscle tissue samples were collected from SBT landed by the Indonesian longline fishery in Bali. Muscle tissue samples were also collected from harvested SBT at tuna processors in Port Lincoln, South Australia. Genotyping of muscle samples from the 2014/15 season is also currently underway for use in future close-kin mark-recapture estimates of spawning stock biomass.

Length and age frequency data from the Indonesian longline fishery shows that since the 2012/13 spawning season, the proportion of small/young SBT (<160 cm FL/12 years old) in the catch landed in Bali has increased substantially compared to previous years. Investigations have shown that SBT caught by Indonesia have occurred in CCSBT statistical areas 1, 2 and 8, so it is plausible that the small/young SBT in the monitoring series were caught south of the SBT spawning ground. Efforts to clarify which fish in the monitoring series were actually caught on the spawning ground (as opposed to, for example, targeted fishing for SBT to the in areas 2 and 8) are ongoing. At this stage it is not possible to identify the catch location of individual SBT sampled as part of the regular the catch monitoring program.

An age calibration/estimation workshop continues to be a priority for consideration in 2017. Indonesia’s Research Institute for Tuna Fisheries (RITF) in Bali was identified to host the workshop. Significant progress has been made at RITF to establish an otolith preparation laboratory for ongoing capacity development in tuna ageing methods.

2 Introduction

Southern bluefin tuna spawn from September to April in an area between Indonesia and the northwest coast of Australia (Farley and Davis, 1998). An Indonesian-based longline fishery operates on this spawning ground year-round targeting yellowfin and bigeye tuna, with a bycatch of SBT. Obtaining an accurate estimate of the size and age composition of SBT landed by the Indonesian longline fishery is an important input for population modelling and stock assessments, and as an indicator to monitor changes in the spawning population over time.

Since the early 1990s, the size and age structure of the SBT spawning population has been monitored through a series of collaborative research programs between CSIRO, Indonesia’s

Research Centre for Capture Fisheries[1] (RCCF) and Research Institute for Marine Fisheries (RIMF), the Indian Ocean Tuna Commission (IOTC), and Japan's Overseas Fishery Cooperation Foundation (OFCF). The program monitors the catch of SBT by Indonesia's longline fleet operating in the north-east Indian Ocean. Initially, the program collected data on SBT landed at the port of Benoa in Bali, but in 2002 this was expanded to include the ports of Muara Baru (Jakarta) and Cilacap (south coast Central Java), and to comply with IOTC monitoring protocols. The majority of targeted SBT sampling, however, still occurs at Benoa, as this is the port where the large majority of SBT are landed.

In 2006, the catch monitoring program in Indonesia was expanded to include the collection of muscle tissue samples for a project to obtain a fishery-independent estimate of spawning biomass of SBT using close-kin (CK) genetic techniques. Tissue samples were also collected from juvenile SBT in Australia over the same period. The project analysed over 13,000tissue samples and was successfully completed in 2013 (Bravington et al. 2014). Since then, tissue sampling has been ongoing with approximately 3200 (1600 juveniles and 1600 adults) samples collected and archived annually.

In 2013, the Extended Scientific Committee (ESC) developed a new Scientific Research Plan for southern bluefin tuna (SBT). The SRP was reviewed in 2014 and again in 2015. Several items were identified as high priority in the 2016 work plan including the ageing of Indonesian SBT otoliths and continued collection and archiving of tissue samples for close-kin genetics (Anon 2015). An age calibration/estimation workshop was also a high priority for consideration in 2017. In this paper we provide an update on progress in these activities.

3 Otolith and CK tissue sampling

3.1 Indonesia

As in previous years, targeted sampling of SBT occurred at Benoa Fishing Port in the 2015/16 spawning season using the existing Indonesia-CSIRO monitoring system for the longline fishery (e.g. see Proctor et al., 2006). Length measurements, otoliths and muscle tissue samples were obtained for 2000 SBT ranging in size from 116 to 200 cm fork length (FL)(Table 1).The number of SBT sampled was slightly higher than originally planned (n=1600) to allow sampling to continue through to the end of the season, as 1600 had already been collected by early February.

Otoliths were collected, cleaned/dried and placed in vials. Muscle tissue samples were collected and frozen. Sex was identified for all fish based on residual gonad tissue. Approximately half of the otoliths have been received in Hobart and the remaining otoliths and muscle samples will be transported to CSIRO for archiving as soon as possible.

[1] RCCF subsequently became *Research Center for Fisheries Management and Conservation*, and more recently, *Center for Fisheries Research and Development*.

3.2 Australia

Muscle tissue samples were also collected from juvenile SBT in June-July 2016 at the tuna processors in Port Lincoln, South Australia. Muscle tissue was collected and frozen according to protocols provided by CSIRO. Tissue was obtained from 1600 fish ranging from 103 to 109 cm FL (age 3 fish). The tissue will be transported to CSIRO in Hobart in late August. The muscle samples are stored in consecutively labelled boxes with 100 positions (10 by 10) in each box (A01 through J10). Individual sample are given a unique identification label (e.g., SbPL2014_Bx01_A01) and will be placed in either -80 or -20°C for long term storage.

4 Ageing Indonesian SBT

4.1 Methods

Direct ageing of a subsample of 500 otoliths was undertaken for fish sampled in the 2014/15 spawning season (Table 1). Otoliths were selected based on size of fish (length stratified sampling scheme rather than random sampling) to obtain as many age estimates from length classes where sample sizes were small. Length stratified sampling is the best way of obtaining sufficient age estimates from length classes where sample sizes are small, while providing enough estimates for each season. Otoliths were sent to Fish Ageing Services Pty Ltd (FAS) for sectioning and reading using the techniques described in Anon. (2002). The otolith reader has at least 10 years' experience reading SBT otoliths.

Each otolith was read twice by the primary otolith reader (FAS) and then a final age estimate was given to 482 fish. All readings were conducted without reference to the size of the fish, date of capture, or to previous readings. The precision of readings was calculated using Average Percent Error (Beamish and Fournier, 1981).

To determine the age structure of the Indonesian catch of SBT in the 2014/15 season, an age-length key (ALK) was developed using the sample of aged fish. The age-length-key gives the proportion of fish at age in each 5-cm length class, which enabled us to infer the age-frequency distribution of the catch from the length-frequency distribution obtained through the monitoring. This method has been used to estimate the age distribution of the Indonesian catch since the mid-1990s, apart from 2011/12 when no direct age estimates were available. For that season, an ALK was developed using direct age data for the two preceding spawning seasons and applied to the 2011/12 length frequency data.

Table 1. Number of length measurements and age estimates for SBT by spawning season.

SPAWNING SEASON	FORK LENGTH (CM)			OTOLITHS	AGE (YEARS)	
	N	**MEAN**	**RANGE**	***N***	***N*[1]**	**MEAN**
1993/94	676	188.1	161-207	0	0	NA
1994/95	1610	180.7	147-221	549	486	21.2
1995/96	1107	178.9	149-216	225	50	NA
1996/97	1615	179.6	146-218	602	475	20.8
1997/98	1577	176.4	143-214	519	485	19.8
1998/99	936	179.9	145-210	660	474	20.7
1999/00	786	177.4	150-216	533	498	19.5
2000/01	762	174.2	140-210	720	481	16.9
2001/02	821	169.5	147-223	715	489	14.8
2002/03	1385	166.8	134-229	1502	488	14.5
2003/04	1279	168.5	145-215	1283	494	15.2
2004/05	1580	170.1	89-205	1523	493	15.3
2005/06	1182	169.2	122-201	1180	486	14.4
2006/07	1586	168.3	134-202	1586	491	15.1
2007/08	1693	169.5	145-203	1709	485	16.7
2008/09	1704	171.0	143-219	1697	479	15.6
2009/10	1583	168.5	141-204	1538	488	15.3
2010/11	1015	170.4	142-198	1009	481	16.8
2011/12	565	169.4	136-212	543	*NA*	16.0
2012/13	1381	162.1	135-211	1373	474	13.2
2013/14	1810	161.8	100-204	1637	473	13.9
2014/15	3443	161.4	95-225	1346	482	14.4
2015/16	2000	161.0	116-200	2000	NA	NA
Total	32096			22449	9251	*Total*

[1]A random sub-sample of 500 are selected for ageing, apart from the 2011/12 season where an ALK based on data from the previous two seasons was used.

4.2 Results

4.2.1 Length distribution

Figure 1 shows the length frequency distributions for SBT caught by the Indonesian longline fishery by spawning season. The data are separated into those caught on and those caught just south of the spawning ground in the 2003/04 to 2006/07 seasons (see Farley et al., 2007) as SBT caught south of the spawning ground are not considered part of the spawning population. In the mid-2000s, at least one Benoa-based fishing company (Processor A) was identified as having shifted their operations to target SBT south of the SBT spawning ground (Andamari et al., 2005; Proctor et al., 2006; Farley et al., 2007). A greater proportion of the catch landed at Processor A comprised small (<165 cm FL) fish compared to the other processors. SBT of these sizes are consistent with historic Japanese catch data for vessels operating on the staging ('Oki') fishing ground (inside CCSBT Area 2) to the south of the spawning ground (Shingu, 1978).

As noted in previous reports to CCSBT-ESC, considerable change has occurred in the size distribution of SBT caught on the spawning ground since monitoring began. In the mid- and late-1990s, the majority of SBT caught were between 165 and 190 cm FL with a median length of ~180 cm (Fig. 1). In the early-2000s, the relative proportion of small SBT (155-165 cm FL) in the catch increased (Fig. 2). The mean size of SBT caught declined from 188.1 to 166.8 cm between 1993/94 and 2002/03, and remained between 168.3 and 171.0 cm until 2011/12 (Table 1).

In the 2012/13 to 2014/15 spawning seasons, the length frequencies indicate a new mode of very small fish (relative to the historical distribution)between 140 and 155 cm FL in the catch in addition to the "usual" mode around 160-180 cm FL. In these seasons, the proportion of fish <155 cm was between 32.9% and 35.3% compared to much lower levels of 0 to 12.4% in the previous seasons (Fig. 2). This change in the size distribution is reflected in a decrease in the mean size of SBT in the catch to ~162 cm FL (Table 1; Fig. 3).

In 2015/16, small fish were again present in the catch sampled by the monitoring program and the relative abundance of fish <155 cm was32.4% and the mean size was 160.1 cm (Table 1; Fig. 3). However, in this recent period the size distribution is unimodal around 150 to 160 cm FL, rather than bimodal.

4.2.2 Age distribution

A final age was available for 482 of the 500 otoliths selected from the 2014/15 spawning season. Fish ranged in size from 129-201 cm LCF and age estimates ranged from 6 to 31 years. The average percent error between readings was2.74% and the percent agreement was 74.0%. When successive readings of otoliths differed, 97.5% were by ±2 years, indicating a good level of precision.

Figure 4 shows the estimated age structure of the Indonesian catch by spawning season. As expected given the change in the length distribution of the catch, the proportion of young fish aged 10-15 years increased markedly in the early 2000s (Fig. 4). These young fish can be tracked through the age distribution of subsequent years suggesting a pulse of recruitment to the spawning population. A second recruitment pulse of young fish occurred in the mid-2000s (Farley et al. 2014).In 2012/13 and 2013/14, however, there was a substantial increase in the catch of young SBT (7-10 years) (Fig. 4; fig. 5). As expected, the mean age of SBT sampled decreased from 15-16 to 13-14 years (Table 1; Fig.6). The mean age of SBT >20 years has also decreased since the mid-2000s (Fig. 6). In 2014/15, the young SBT observed in recent years have persisted in the catch.

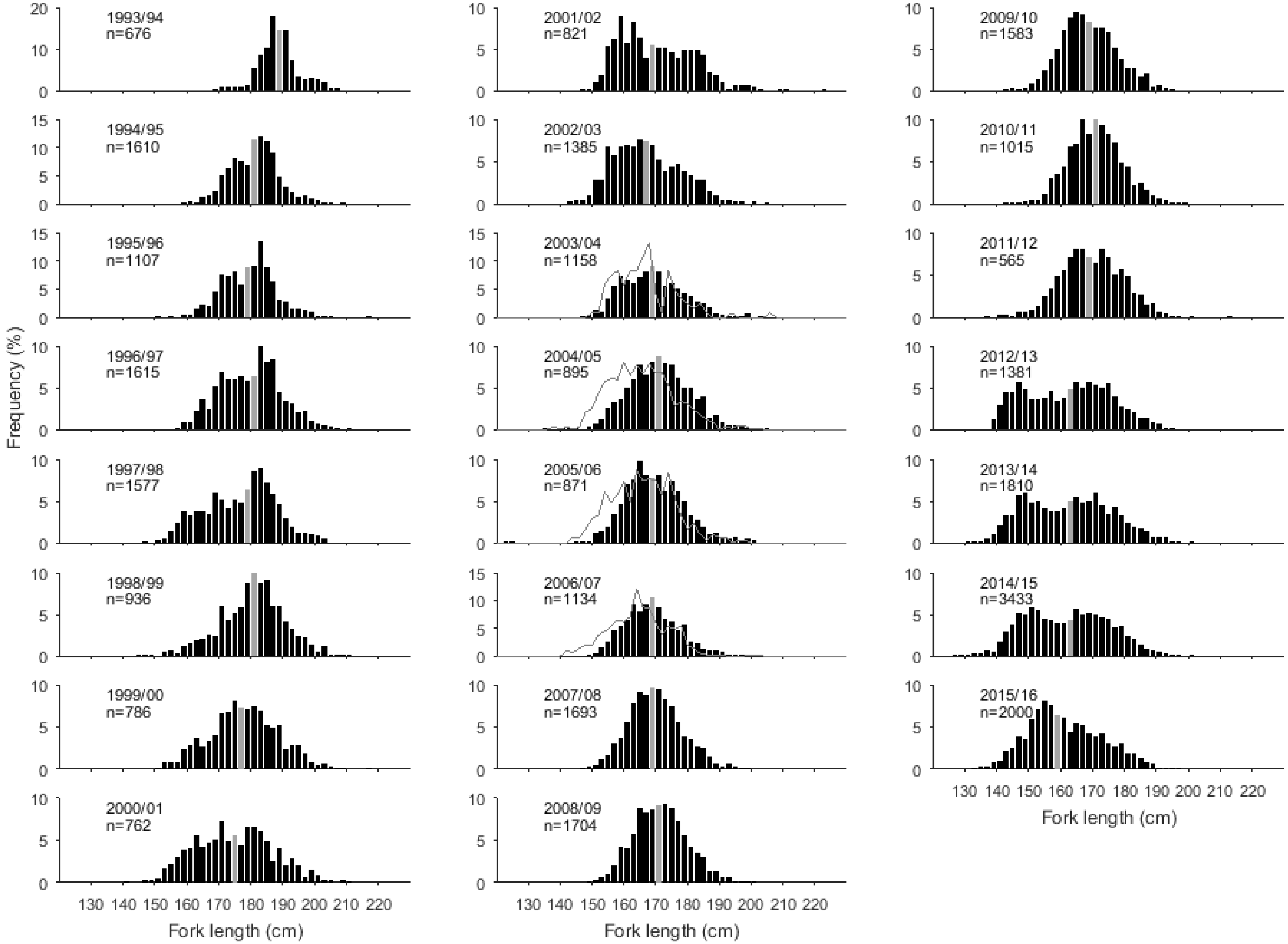

Figure 1. Length frequency (2 cm intervals) of SBT caught by the Indonesian longline fishery (bars) by spawning season. The grey bar shows the median size class. For comparison, the length distribution of SBT thought to be caught south of the spawning ground (Processor A) is shown for the 2003/04 (n=121), 2004/05 (n=685), 2005/06 (n=311) and 2006/07 (n=452) seasons (grey line) (see Farley et al., 2007). Note that 8 fish between 100 and 129 cm FL were also sampled in 2013/14 but are not shown.

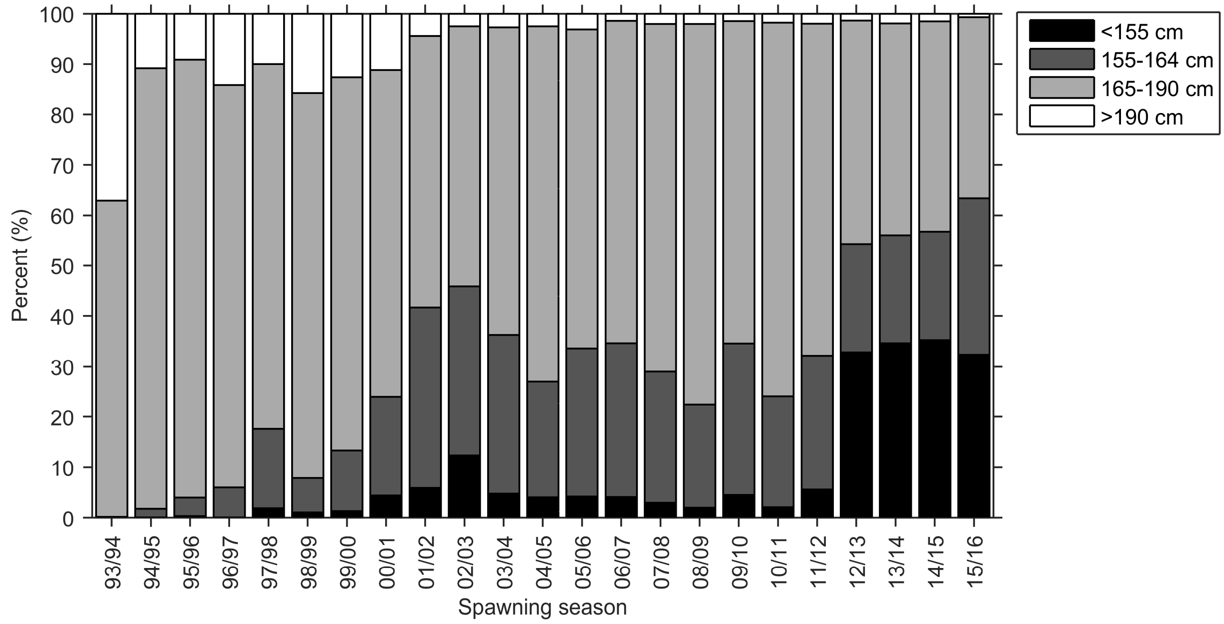

Figure 2. Proportion of SBT caught by the Indonesian longline fishery by size class. Data from Processor A in 2003/04 to 2006/07 are excluded.

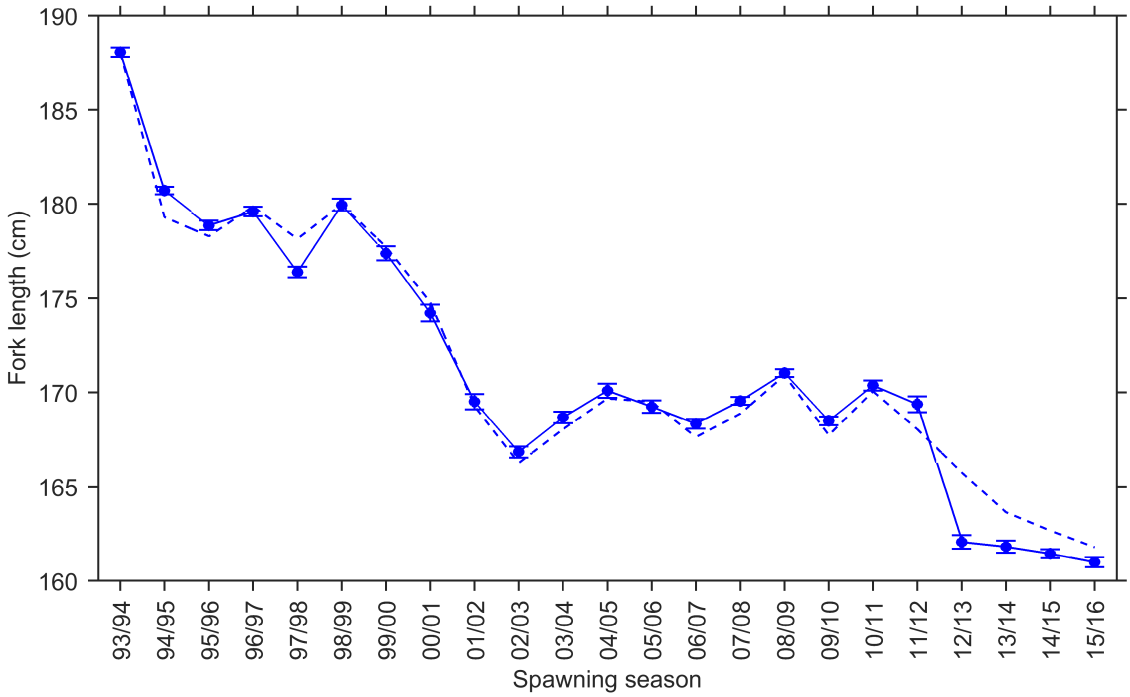

Figure 3. Mean length (+/- 95% CI) of SBT landed by the Indonesian longline fishery by season. Data from Processor A in 2003/04 to 2006/07 are excluded. Dashed line is the mean length of SBT caught in December to May only.

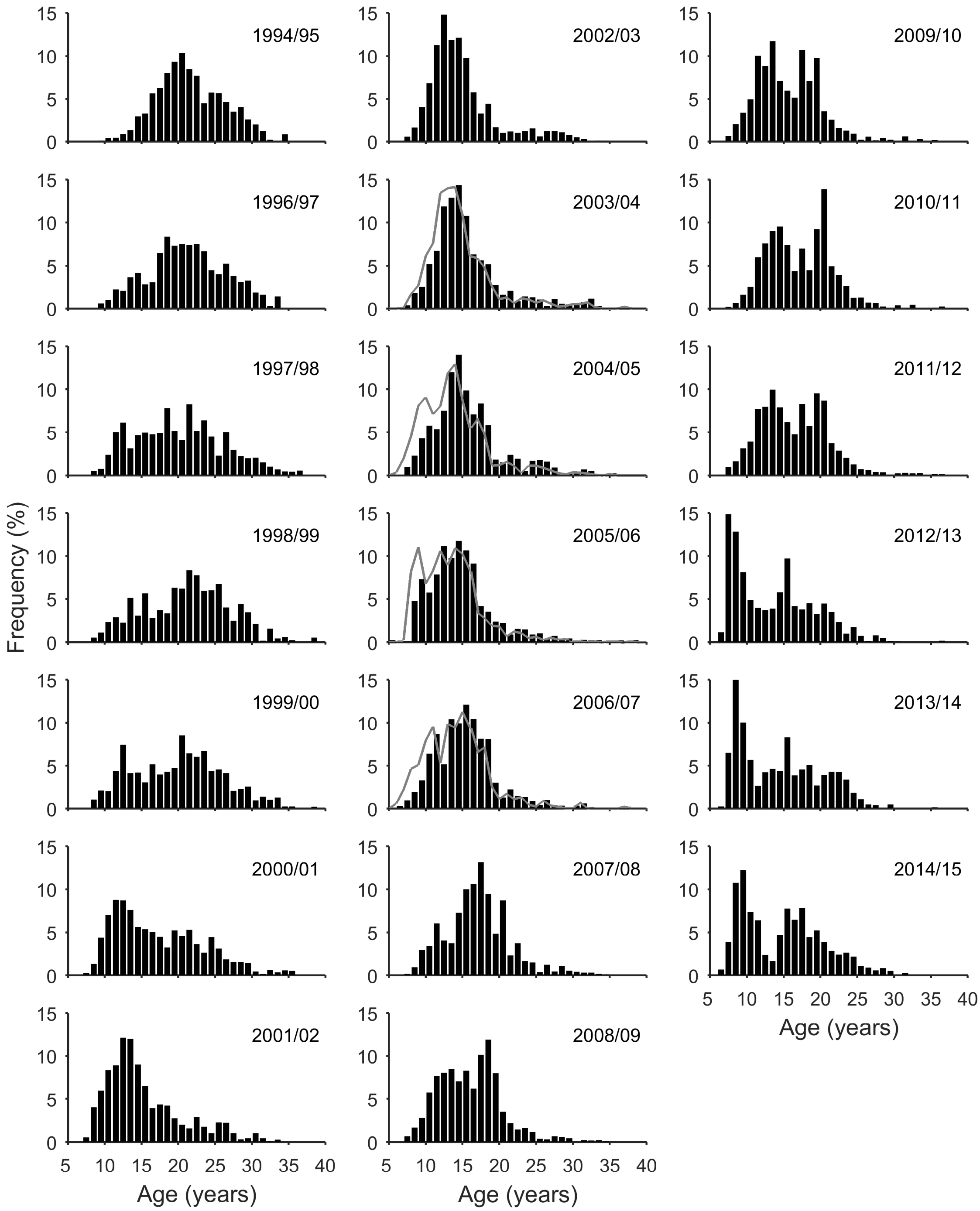

Figure 4. Age frequency distribution of SBT in the Indonesian catch on the spawning ground by spawning season estimated using age-length keys from our sub-samples of aged fish and length frequency data obtained through the Indonesian monitoring program. There was no direct ageing of the 2012–13 otoliths; age frequency is based on the age-length key from the previous two seasons and 2012–13 length frequency data. For comparison, the age

distribution of SBT caught south of the spawning ground (Processor A) is shown for the 2003/04, 2004/05, 2005/06 and 2006/07 seasons (grey line).

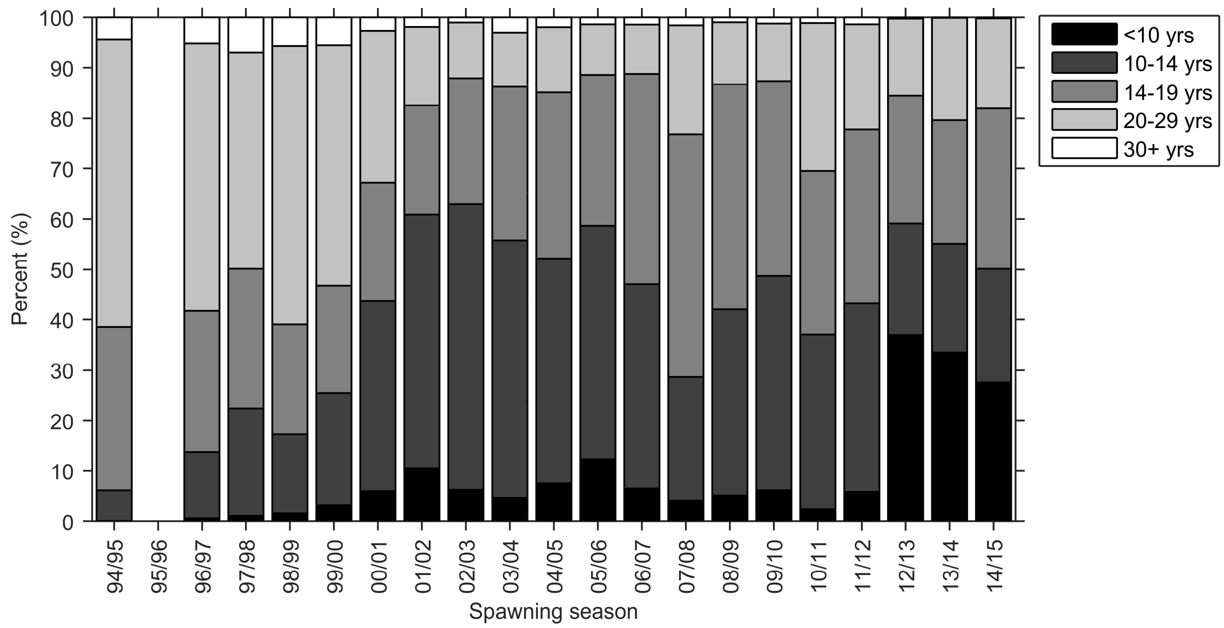

Figure 5. Estimated proportion of SBT by age class in the Indonesian catch. Data from Processor A for 2003/04 to 2006/07 are excluded. Note there are no age data for the 1995/96 season.

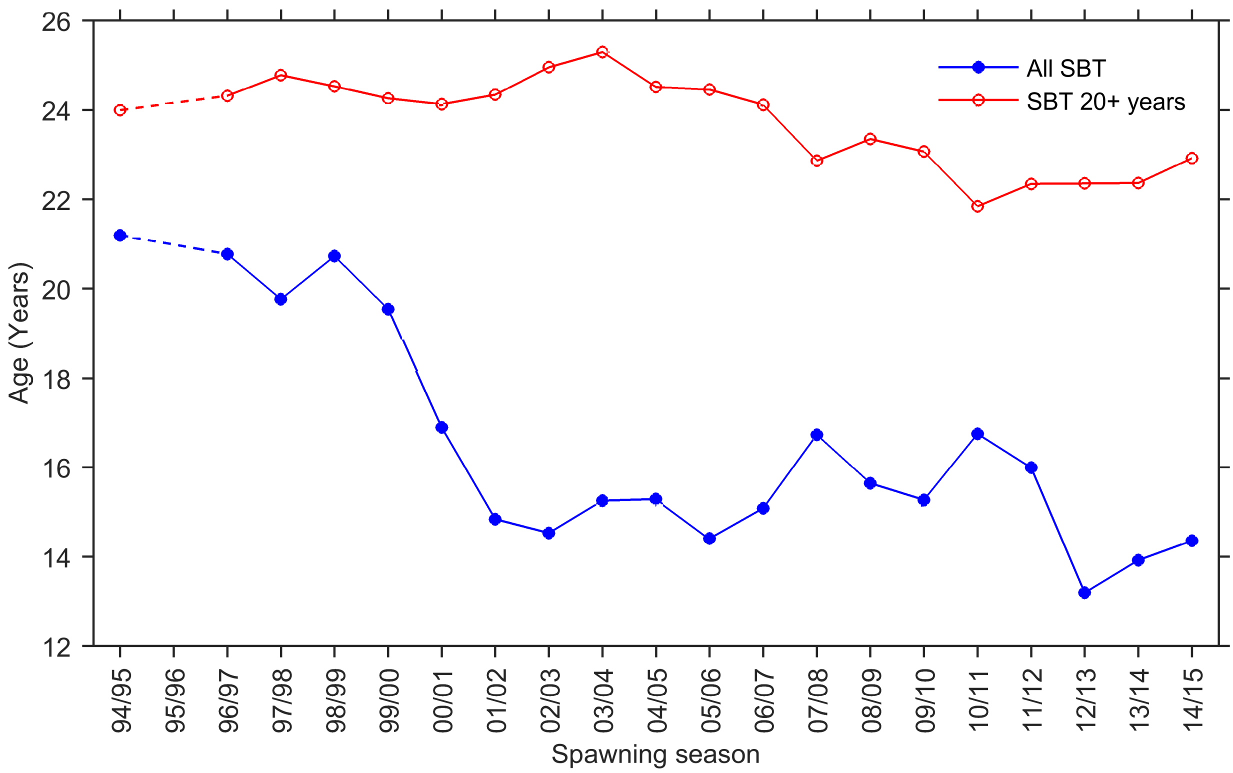

Figure 6. Estimated mean age of SBT in the Indonesian catch. Data from Processor A for 2003/04 to 2006/07 are excluded. Note there are no age data for the 1995/96 season.

4.2.3 Catch locations

Early investigations suggest that the small/young SBT appearing in the Indonesian catch since the 2012/13/14 were likely to have been caught south of the SBT spawning ground. In early May 2014, tuna fishing industry in Benoa participated in a workshop[2] at which a presentation was given highlighting the importance of catch location information to enable a better understanding of the increase in smaller fish in the landings. Follow-up discussions at the Benoa office[3] with responsibility for monitoring of fishing vessel activity revealed that VMS is available for some of the Benoa longline fleet and may provide validation of catch location information for the smaller SBT, should such information be provided by individual fishing companies.

Further investigation has shown that Indonesian catches of SBT have occurred in CCSBT statistical areas 1, 2 and 8. Investigations are ongoing but at this stage, it is not possible to identify the catch location of individual SBT sampled in the catch monitoring program.

4.3 Age estimation workshop

Farley et al. (2015) reiterated the requirements for an ageing calibration/estimation workshop for SBT and Indonesia's Research Institute for Tuna Fisheries (RITF) in Bali was identified to host the workshop. A workshop is required to examine the precision and bias of age estimates between readers and among laboratories to maintain a consistent level of precision and minimise the potential for systematic biases in ageing estimates

Over the past 12 months, significant work has been undertaken at RITF to establish a new otolith preparation laboratory, which is nearing completion. The lab will be available for ongoing capacity development in tuna ageing methods which should ultimately put Indonesia in a position to undertake the annual sectioning and reading (ageing) of SBT otoliths.

5 Close kin genotyping

A total of 2000 close-kin muscle tissue samples collected in the 2014/15 season were selected for genotyping. Of these, 1000 were from fish caught by the Australian surface fishery in the Great Australian Bight (juveniles) and 1000 from fish landed in Bali, Indonesia. Only fish ≥145 cm FL were included in the Indonesian samples to avoid samples unlikely to be from mature fish. The samples for analysis were selected randomly from the pool of samples collected in each region.

DNA was extracted from a 10mg sub-sample of tissue from all 2000 individuals. A bead-based extraction protocol (Machery Nagel Nucleomag) kit was used on an Eppendorf EP motion robot to produce a 150uL archive and 50uL working stock of DNA in micro-titre format plates. Archive

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[2] Workshop "Monitoring and Evaluation of Enumerator and Observer Acitivties in Port of Benoa, Bali", 5 May 2014, held at office of Asosiasi Tuna Longline Indonesia, Benoa.

[3] Pelabuhan Perikanan Nusantara Pengambengan – Benoa Office, a regional office under Directorate General of Capture Fisheries.

plates of extracted DNA are stored at dedicated -80’C freezers located at CSIRO Hobart. Working stock plates of extracted DNA were shipped to Diversity Arrays Technology (DArT) in Canberra for genotype sequencing of approximately 2000 single nucleotide polymorphic loci (SNPs). When complete, the sequencing information will be transmitted to CSIRO Hobart where DNA profiles will be compiled for future downstream analysis to identify kin genotype relationships.

6 Summary

The collection of such large quantities of length frequency data, and the development of validated methods to directly age SBT using the otoliths sampled, have allowed us to accurately estimate the age composition of the Indonesian catch. These data have shown that the spawning stock of SBT has undergone substantial changes since monitoring began; the greatest change being a shift in the mode of SBT caught from 18-22 years in the mid-1990s to 12-15 years in the early-2000s. This increase in the catch of young fish in the early-2000s can be tracked as a pulse of fish through subsequent years. A second pulse of young fish is apparent in the mid-2000s.

Over the last four spawning seasons, a relatively large proportion of SBT landed, compared to previous seasons, were very small/young (<160 cm FL/12 years old). Initial investigations indicate that a proportion of these fish are likely to have been caught on or south of the SBT spawning ground and reflect the development of targeted fishing for SBT and/or albacore in Areas 2 and 8. At this stage, however, it is not possible to definitively identify those fish from the catch monitoring program that were caught on the spawning ground from those that were likely caught to the south with the information sources available.

It is important that we understand where the small fish are being caught because of how these data are used in the SBT operating model. The Indonesian age frequency (from direct aging) are used in the SBT operating models and the fishery selectivity estimates from the operating models are used in projections and used to test the management procedure. Substantial changes in selectivity in a fishery could trigger exceptional circumstances under the SBT MP meta-rules process, because the MP has not been tested under these conditions. If the small fish are coming from locations to the south of the spawning ground (Area 1), then these data may need to be assigned to a different fishery within the SBT OM fishery definitions (Anon 2014), e.g. Area 2 or 8, based on current information.

The Indonesian monitoring data are also used in the current close-kin (CK) estimation framework. It is assumed that all catches come from individuals that were mature and spawning in the year they were sampled. Hence, these recent changes also have the potential to influence how these data can be used in the future in the CK abundance estimation.

The collection of CK samples in 2015/16 and genotyping of samples from the 2014/15 season will allow for future direct monitoring of spawning stock biomass.

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