



# 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.....  | 1  |
| 2 | Introduction.....  | 1  |
| 3 | Methods.....   | 2  |
|   | 3.1 ..... Otolith and tissue collection.....                 | 2  |
|   | 3.2 ..... Indonesian SBT ageing.....                         | 4  |
|   | 3.3 ..... Close kin genotyping.....                          | 4  |
| 4 | Results .....  | 5  |
|   | 4.1 ..... Size/age composition of the Indonesian catch ..... | 5  |
|   | 4.2 ..... Close kin genotype data .....                      | 10 |
| 5 | Summary.....   | 11 |
|   | References .....   | 12 |

# 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.

Otoliths and muscle tissue samples were collected from SBT landed by the Indonesian longline fishery in Bali during the 2016/17 spawning season (1 July to 30 June). Muscle tissue samples were also collected from harvested SBT at tuna processors in Port Lincoln, South Australia, in 2017.

Genotyping of muscle samples from the 2015/16 season is currently underway for use in future close-kin mark-recapture estimates of spawning stock biomass. DNA extracted from muscle samples collected in the previous season in Indonesia (2014/15) was of poor quality and it was agreed that the 2010 adults would be genotyped in lieu of the 2015 samples.

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. The data indicates that the mode of small fish has progressed through the fishery over the past 5 years, which is also observed in the New Zealand charter fleet catch data. 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. 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.

## 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 accurate estimates of the size and age composition of SBT landed by the fishery, as well as estimates of adult abundance, are important for population modelling and stock assessments, and as an indicator to monitor changes in the spawning population over time.

The early 1990s marked the beginning of what is now 25 years continuous monitoring of the size and age structure of the SBT spawning population. This has been done through a series of collaborative research programs between CSIRO and Indonesia's marine fisheries research

institutes<sup>1</sup> within the Ministry of Marine Affairs and Fisheries (MMAF). Initially the focus of monitoring was only on the SBT in the landings and only at Benoa Fishing Port (Bali) where the majority of the vessels that fish on the SBT spawning ground were, and still are, based. In 2002, in collaboration with the Indian Ocean Tuna Commission (IOTC) and Japan's Overseas Fishery Cooperation Foundation (OFCF), the program expanded to include enumeration of landings at the ports of Muara Baru (Jakarta) and Cilacap (south coast Central Java). In following IOTC monitoring protocol the program also expanded to include enumeration of all species in the landings; tunas (the primary target species of yellowfin and bigeye tuna, SBT, and albacore), billfish, and other bycatch species. The majority of targeted SBT sampling, however, still occurs at Benoa, as this is the port where the majority of Indonesian caught SBT are landed.

Prior to 2006 the monitoring program included the sampling of otoliths from the 'reject' (graded as non-export quality) SBT. In 2006 the catch monitoring program was expanded to include the collection of muscle tissue samples, from the same fish sampled for otoliths, for a project to obtain a fishery-independent estimate of SBT spawning biomass using close-kin mark-recapture (CKMR) genetic techniques. Tissue samples were also collected from juvenile SBT in Australia over the same period. The project analysed over 13,000 tissue samples and was successfully completed in 2013 (Bravington et al. 2014; 2016). Since then, tissue sampling has been ongoing with approximately 3200 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). In this paper we provide an update on progress in these activities.

## 3 Methods

### 3.1 Otolith and tissue collection

In Indonesia, targeted sampling of SBT occurred at Benoa Fishing Port in the 2016/17 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 1500 SBT ranging from 149-180 cm fork length (FL) (Table 1). Otoliths were collected, cleaned/dried and placed in vials. Muscle tissue samples were collected, placed in vials and frozen. Sex was identified for all fish based on residual gonad tissue.

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<sup>1</sup> Indonesian collaborating institutions, in chronological order: Central Research Institute for Fisheries Indonesia (CRIFI), Research Institute for Marine Fisheries (RIMF), Research Centre for Capture Fisheries (RCCF), Research Centre for Fisheries Management and Conservation (RCFMC), and currently Centre for Fisheries Research (CFR) in Jakarta, and Research Institute for Tuna Fisheries (RITF) in Bali.

In addition, length data were obtained from the daily monitoring by RITF enumerators of the longline vessel landings in Benoa (Irianto et al. 2016), for 2012 to 2016.

In Australia, muscle tissue samples were collected from juvenile SBT in June-July 2017 at the tuna processors in Port Lincoln, South Australia. Tissue was obtained from 1500 fish ranging from 98 to 109 cm FL. This size range samples is slightly wider than sampled for the past four years (103-109 cm), to endure the full size range of 3 year-olds is being sampled. The muscle tissue was frozen according to protocols provided by CSIRO.

All otoliths and tissue samples have been transported to CSIRO in Hobart. The otoliths are stored dry. The muscle samples are stored frozen 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 longer term storage.

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

| SPAWNING SEASON      | FORK LENGTH (CM) |       |         | OTOLITHS N   | AGE (YEARS)    |              |
|----------------------|------------------|-------|---------|--------------|----------------|--------------|
|                      | N                | MEAN  | RANGE   |              | N <sup>1</sup> | 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              | 864              | 170.5 | 136-212 | 543          | NA             | 16.3         |
| 2012/13              | 2051             | 164.1 | 131-211 | 1373         | 474            | 14.2         |
| 2013/14              | 1905             | 161.7 | 100-210 | 1637         | 473            | 13.8         |
| 2014/15              | 2774             | 159.9 | 95-225  | 1346         | 482            | 13.8         |
| 2015/16              | 2925             | 162.7 | 119-210 | 2000         | 477            | 13.8         |
| 2016/17 <sup>2</sup> | 1500             | 163.3 | 149-180 | 1500         | 0              | NA           |
| <b>Total</b>         | <b>34261</b>     |       |         | <b>22449</b> | <b>9251</b>    | <b>Total</b> |

<sup>1</sup>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.

<sup>2</sup> Preliminary data only.

## 3.2 Indonesian SBT ageing

Direct ageing of a subsample of 500 otoliths was undertaken for fish sampled in the 2015/16 spawning season (Table 1). As for previous years, 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 477 fish. The remaining 23 otoliths could not be read. 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 2015/16 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.

## 3.3 Close kin genotyping

### 3.3.1 2014/15 tissue samples

Farley et al (2016) reported that 2024 close-kin muscle tissue samples that were collected in the 2014/15 season were selected for genotype sequencing. DNA was extracted from all samples (juveniles and adults) and DNA from the juveniles were sent for genotype sequencing (n=1012).

Prior to sequencing the adult samples, a check of the DNA quality was undertaken as part of standard quality control checks. This showed that quality and quantity of the DNA from the adult 2015 samples was poor and less than 10 % showed reasonable potential for successful DNA sequencing. As a result, none of the 2015 adults were genotyped, as the majority would be rejected by the sequencing facility. The likely cause of the poor quality tissue was a freezer break down and/or an electricity blackout at the Benoa Fishing Port prior to samples being transferred to the RITF freezers. We did not find similar tissue degradation in any of the other sampling year.

The lack of the suitable 2015 adult samples will reduce the number of adults available for comparison with the juveniles and, as a result, the overall number of POPs likely to be identified. This is likely to reduce the precision of the final abundance estimate from that specified in the

design study. After discussion with the CCSBT Executive Secretary, it was agreed that the 2010 adults would be genotyped in lieu of the 2015 samples as they could be compared with two cohorts of juveniles (2011 and 2012) and will improve the precision of the time-series of adult SBT abundance that will be used in the 2017 stock assessment (see Bravington et al., 2017).

### **3.3.2 2015/16 tissue samples**

In addition to the above, 2024 muscle tissue samples collected in the 2015/16 season were selected for genotyping. Again, 1012 were from fish caught by the Australian surface fishery in the Great Australian Bight (juveniles) and 1012 from fish landed in Bali, Indonesia. The samples from Australia were selected randomly from the pool of samples collected. The samples from Indonesia were selected based on size of fish. Only fish  $\geq 150$  cm FL were included to avoid samples unlikely to be from mature fish. Of those, all large fish were selected ( $>178$  cm) and a fixed number of samples were selected randomly within each of the remaining 1-cm length class.

DNA was extracted from a 10mg sub-sample of tissue from all fish. For most samples, a magnetic 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. For some samples, a new filter based column extraction protocol (Qiagen) was used.

Archive plates of extracted DNA are stored at dedicated  $-80^{\circ}\text{C}$  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 completed, the sequencing information will be transmitted to CSIRO Hobart.

## **4 Results**

### **4.1 Size/age composition of the Indonesian catch**

#### **4.1.1 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 spawning season, the length frequency 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 (Fig. 1). The proportion of fish <155 cm was 22.5% compared to levels of 0-12% in previous seasons (Fig. 2). This change in the size distribution was reflected in a decrease in the mean size of SBT in the catch (Table 1; Fig. 3).

Figure 1 indicates that the mode of small fish has progressed through the fishery moving from ~146 cm in 2012/13 to ~156 cm in 2016/17 season. The progression of the mode is consistent with the size/age composition data from of the New Zealand charter fleet catch, which showed the same modal progression (Anon, 2016). This may be the result of fish growth over time after strong recruitment in the mid-2000s.

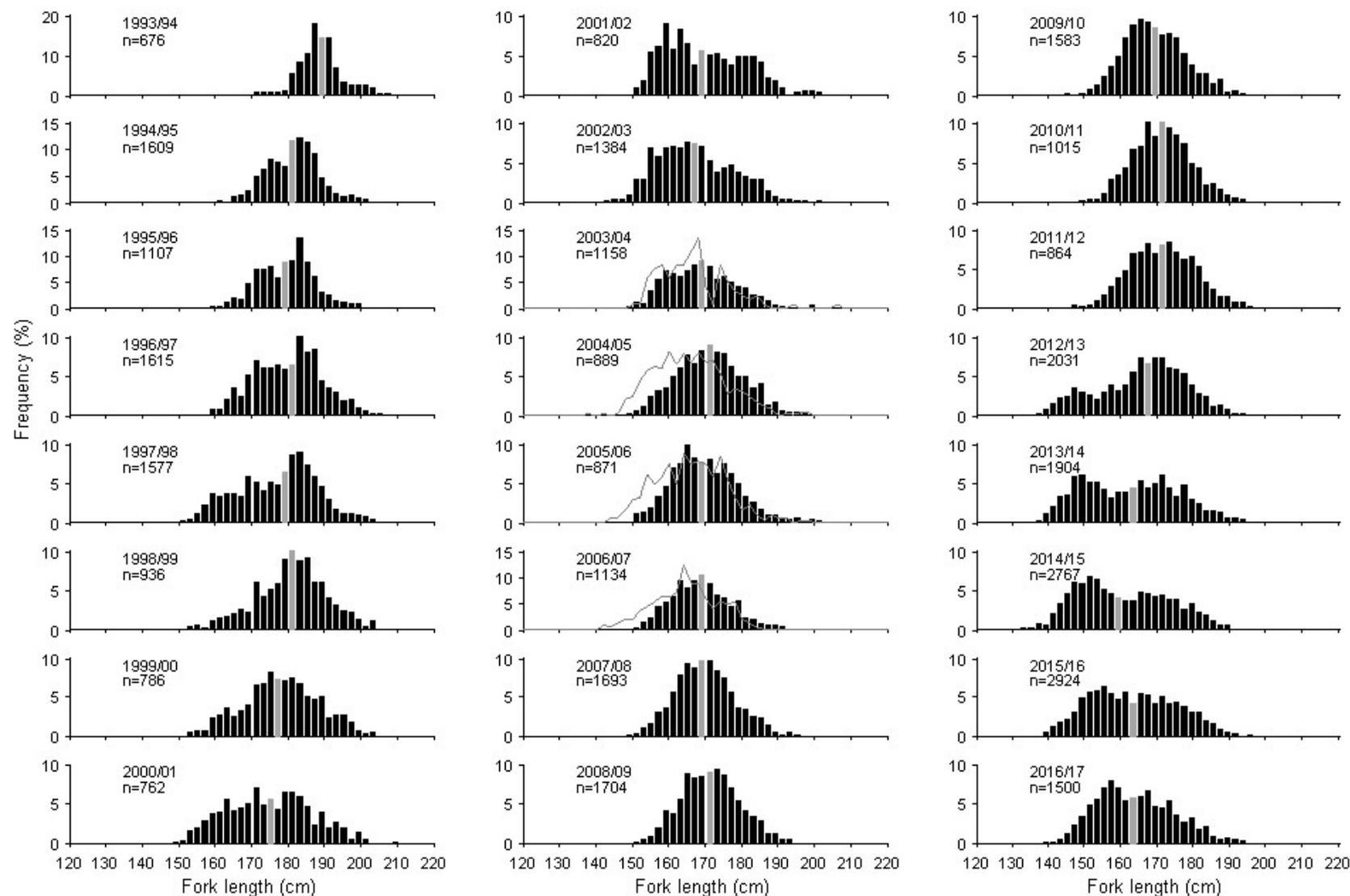
#### **4.1.2 Age distribution**

A final age was obtained for 477 of the 500 otoliths selected from the 2015/16 spawning season. Fish ranged in size from 127-200 cm FL (Table 1) and age estimates ranged from 6 to 32 years. The average percent error between readings was 2.51%. When successive readings of otoliths differed, 97.7% were by  $\pm 2$  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). The mean age of SBT >20 years has decreased since the mid-2000s (Fig. 6).

In 2012/13 there was a substantial increase in the catch of young SBT (<10 years) and the mean age of SBT sampled decreased (Fig. 4-6). The mode of young fish in the catch has persisted each year since that time, and there is some indication that the mode has moved through the fishery on an annual time step.

Earlier investigations suggested that the small/young SBT appearing in the Indonesian catch since 2012/13 were likely to have been caught south of the SBT spawning ground (Farley et al., 2016). Indonesian catches of SBT have occurred in CCSBT statistical areas 1, 2 and 8, but at this stage, it is not possible to identify the catch location of individual SBT sampled in the catch monitoring program.



**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 36 fish <120 cm are not shown and the data for 2016/17 are preliminary.

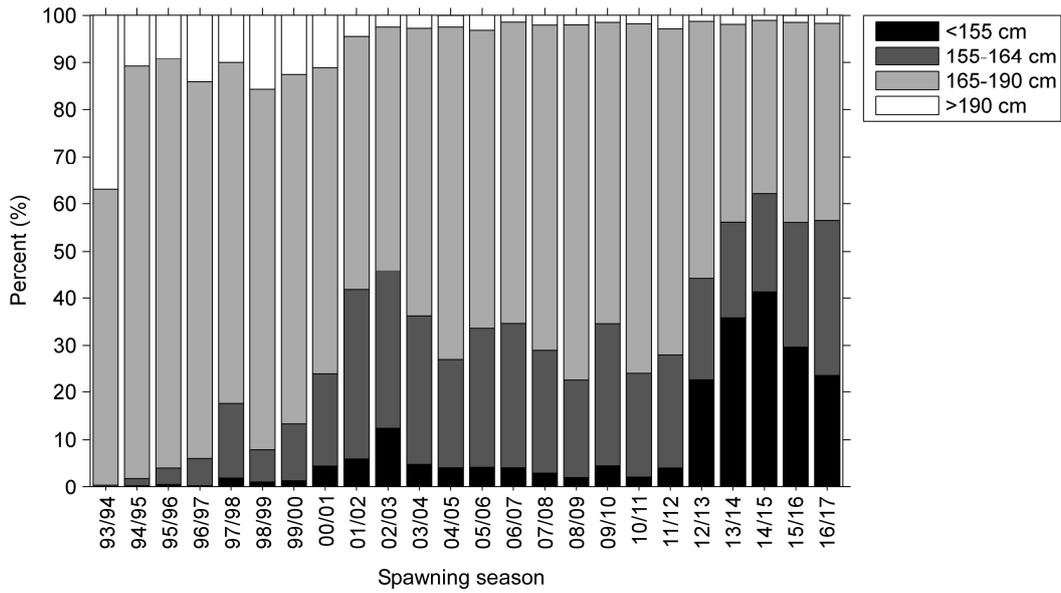


Figure 2. Proportion of SBT caught by the Indonesian longline fishery by size class. Data from Processor A in 2003/04 to 2006/07 are excluded.

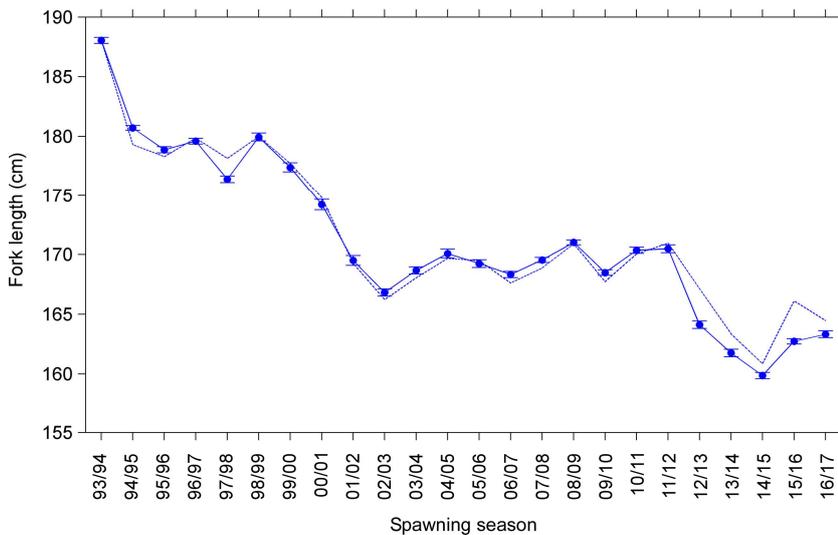


Figure 3. Mean length (+/- 95% CI) of SBT landed by the Indonesian longline fishery by season. Data from Processor A in 2003/04 to 2006/07 are excluded. Dashed line is the mean length of SBT caught in December to May only.

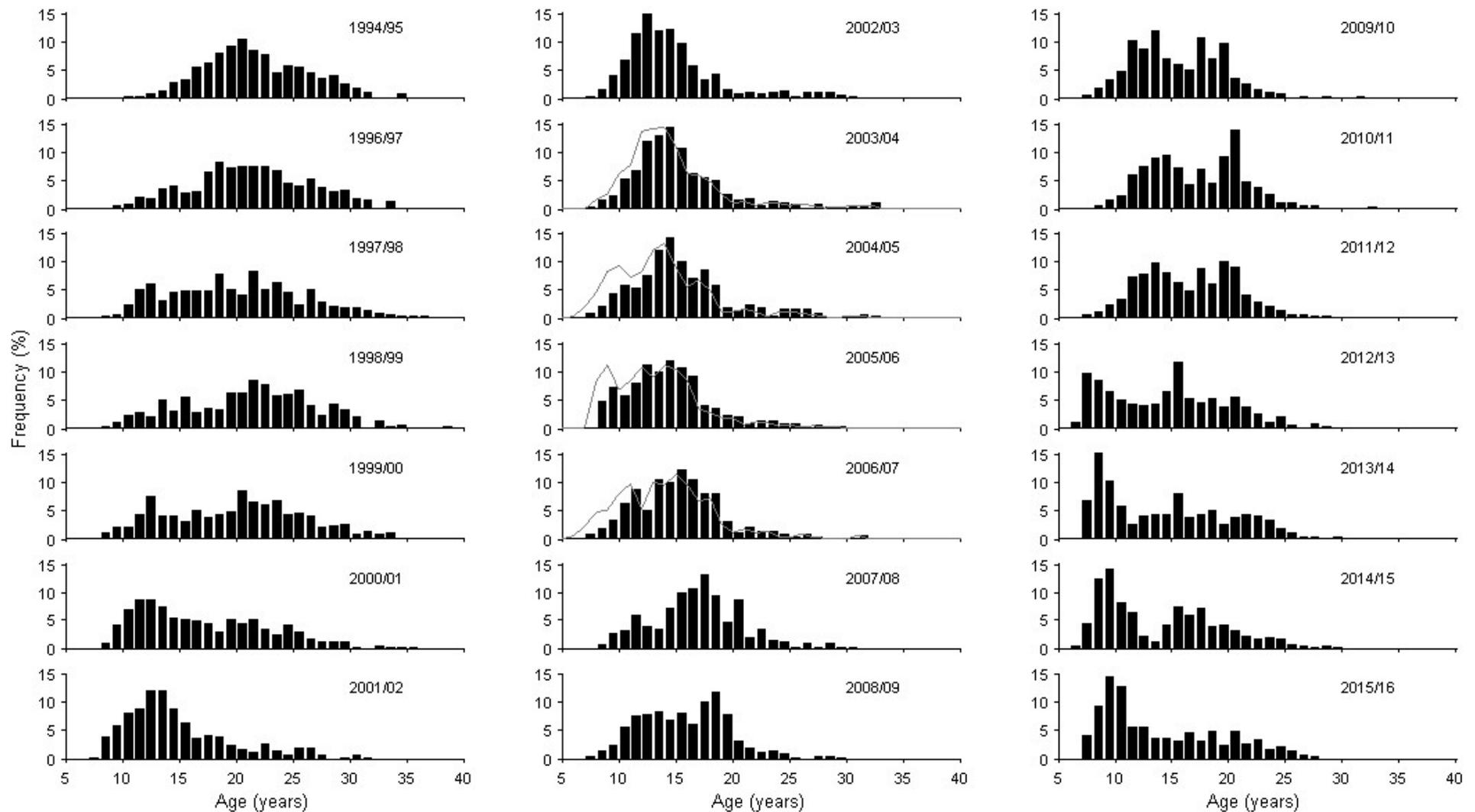


Figure 4. Age frequency distribution of SBT in the Indonesian catch on the spawning ground by spawning season estimated using age-length keys from our sub-samples of aged fish and length frequency data obtained through the Indonesian monitoring program. There was no direct ageing of the 2012–13 otoliths;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

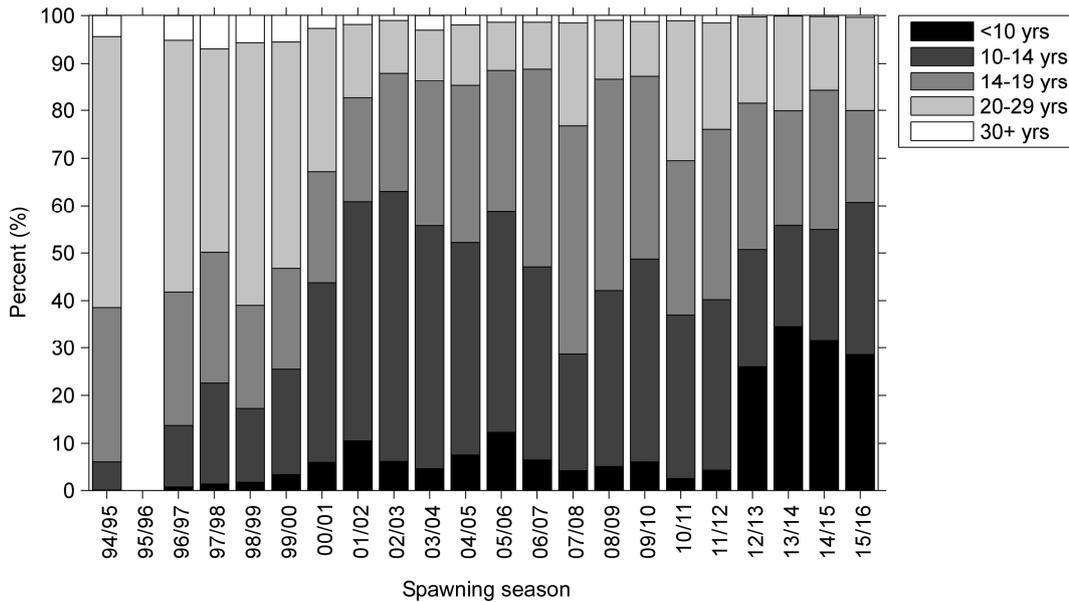


Figure 5. Estimated proportion of SBT by age class in the Indonesian catch. Data from Processor A for 2003/04 to 2006/07 are excluded. Note there are no age data for the 1995/96 season.

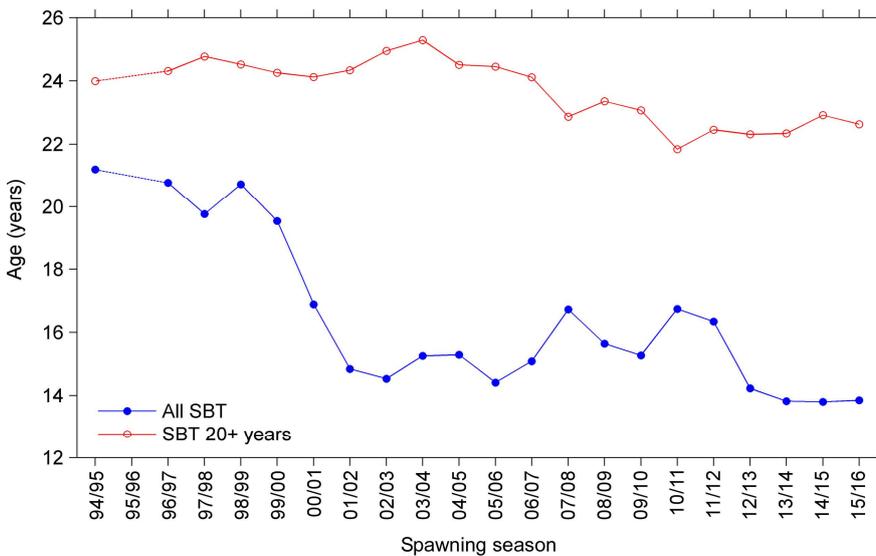


Figure 6. Estimated mean age of SBT in the Indonesian catch. Data from Processor A for 2003/04 to 2006/07 are excluded. Note there are no age data for the 1995/96 season.

## 4.2 Close kin genotype data

Sequencing data were received from DArT for the 2015 juveniles (n=1005) and 2010 adults (n=1012). These data were included in the analysis to estimate a time-series of absolute abundance and mortality of the spawning stock of SBT from 2002 through to 2012 (see Bravington et al. 2017).

## 5 Summary

The collection of such a long time series 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 five spawning seasons, however, a relatively large proportion of SBT landed were very small/young (<160 cm FL/12 years old), compared to previous seasons. 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. We note the importance of the Benoa Observer Program for the collection of at-sea data at SBT catch locations. This, as well as analysis of VMS data and further discussions with fishing companies, may provide validation of catch location information for the smaller fish.

The Indonesian monitoring data are also used in the current close-kin 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 are used in the CK abundance estimation.

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