



# Catch-at-age of Southern Bluefin Tuna in the New Zealand long line fishery 2014/15

New Zealand Fisheries Assessment Report 2017/09

K. Krusic-Golub

ISSN 1179-5352 (online)

ISBN 978-1-77665-494-9 (online)

February 2017



Requests for further copies should be directed to:

Publications Logistics Officer

Ministry for Primary Industries

PO Box 2526

WELLINGTON 6140

Email: [brand@mpi.govt.nz](mailto:brand@mpi.govt.nz)

Telephone: 0800 00 83 33

Facsimile: 04-894 0300

This publication is also available on the Ministry for Primary Industries websites at:

<http://www.mpi.govt.nz/news-and-resources/publications>

<http://fs.fish.govt.nz> go to Document library/Research reports

© Crown Copyright - Ministry for Primary Industries

# Contents

Executive Summary.....	1
Introduction .....	2
Objectives.....	3
Methods .....	3
Otolith Collection.....	3
Sub-sampling of otoliths.....	3
Preparation and ageing .....	3
Birthdate .....	4
Marginal Increment measurement .....	4
Data analysis.....	4
Results .....	5
Otolith data .....	5
Age estimation and precision .....	5
Proportions at age.....	5
Discussion and Conclusion.....	6
Acknowledgements .....	7
References .....	7
Tables .....	8
Figures .....	11
Appendix 1 – Detailed Methods.....	14
Otolith mass.....	14
Preparation of otoliths .....	14
Counts and measurements .....	14
Image collection .....	15
Precision of age estimates .....	15
Appendix 2 – Comparison of length for sub-sampled otoliths and total.....	16
Appendix 3 – Age-otolith weight relationships.....	16
Appendix 4 – Age difference table (Age 1 – Age2).....	17
Appendix 5 – Adjusted age composition 2009–2015.....	18



## Executive Summary

**Krusic-Golub, K. (2017). Catch-at-age of Southern Bluefin Tuna in the New Zealand long line fishery 2014/15.**

*New Zealand Fisheries Assessment Report 2017/09. 18 p.*

This report describes the collection of age data for Southern Bluefin Tuna (*Thunnus maccoyii*) caught in the 2014/15 New Zealand longline fishery. Southern Bluefin Tuna are managed by the Commission for the Conservation of Southern Bluefin Tuna (CCSBT) and form a valuable fishery in New Zealand waters, taken by both domestic and chartered longline vessels. Cohort strength was previously determined using cohort slicing to convert catch-at-length to catch-at-age. However, the CCSBT have recognised that it is preferable to use direct age estimation from otoliths. Therefore, sub-samples of otoliths collected during each fishing season since April 2001 have been aged under various MPI projects and used to determine catch-at-age for each fishing year.

In this project subsamples of otoliths from Southern Bluefin Tuna, routinely collected by observers aboard New Zealand domestic and foreign charter vessels between 26/04/2015 and 20/06/2015 were selected for ageing. Ageing protocols developed at the “Direct Age Estimation Workshop of the CCSTN” held 11–14 June, 2002, in Queenscliff, Australia were followed to provide estimates of age. A total of 255 age estimates were provided to the Ministry for Primary Industries. Age estimates ranged from 2 to 24 years. Repeat readings were produced and results were well within the predetermined acceptable precision limits and indicated a low level of error within the readings.

Proportion-at-age in the catch was estimated by applying the standard age length-key method to the age data and the size frequency distributions obtained from sampling the catch. The results indicated that the age composition for the 2015 (2014/15) season was dominated by age classes 6–10. As in previous years there were very few samples less than 4 years of age in the catch.

## Introduction

Southern Bluefin Tuna (STN) are managed by the Commission for the Conservation of Southern Bluefin Tuna (CCSBT) and form a valuable fishery in New Zealand waters targeted by both domestic and chartered longline vessels.

Given the low current biomass levels for the stock, information on recent year class strength is particularly important. Previously cohort strength had been determined using cohort slicing to convert catch-at-length to catch-at-age, but it was increasingly realised that it is more reliable to use direct age estimation. Therefore, sub-samples of otoliths collected from April 2001 (2000/01 fishing season) through to July 2014 (2013/14) were aged under MPI (formerly MFish) projects IFA2004/03 (Krusic-Golub 2005), STN2006/01 & STN2007/01 (Krusic-Golub 2009), STN2009/01 (Krusic-Golub 2012) and STN2011/01 (Krusic-Golub 2015) to determine catch-at-age for these years. The results of this work were found to be useful for providing indicators of recruitment strength.

The sample size for age estimating for each fishing year is based on estimates described in Morton & Bravington (2003). They concluded that 100–200 per year would be sufficient for the Australian surface fishery, 200 for the Japanese longline fishery, and 500 for the Indonesian fishery. At the beginning of this work (IFA2004/03) 200 fish per year were aged from the New Zealand fishery, and while the patterns in the data were generally consistent across years, it was apparent that an increased number of otoliths would reduce the uncertainty in the proportions of younger ages taken in the catch.

Based on this, a minimum of 250 otoliths are required to be aged from otolith collections from the New Zealand fishery each year. The otoliths sampled for this project were collected during the 2014/15 fishing season, herein referred to as the 2015 season. The report of the “Direct Age Estimation Workshop of the CCSBT” held 11–14 June, 2002, in Queenscliff, Australia form the basis for the protocols employed in this project. These protocols include double blind readings of each otolith and the determination of an agreed final age.

During the 2015 fishing season the onboard fisheries observers trialled a change in otolith storage methodology in which otoliths were placed directly into the otolith envelopes rather than being placed in vials before storage in the envelopes. The vials have previously been thought to provide additional protection for the otoliths as STN otoliths are reasonably brittle. Fish Ageing Services were asked to examine otoliths stored using both methods and provide comment back to MPI whether the change in methodology would affect the numbers of otoliths that could not be processed and aged due to damage.

The main output from this project was to estimate the catch-at-age of STN sampled from vessels fishing in New Zealand in the 2015 season and generate individual age data for the CCSBT age database. As stipulated by the CCSBT, it is a requirement that each otolith is assigned an individual identification number which data including year, month, latitude and longitude of capture, fish length, otolith age estimate, any other relevant comments can be assigned to.

This report describes the collection and analysis of age data provided from project SEA2015-19, and is a continuation of work started under IFA2004/03 to monitor the ages of Southern Bluefin Tuna taken in the New Zealand fishery.

## Objectives

- Age a minimum of 250 otoliths from STN collected by scientific observers aboard vessels fishing in New Zealand fisheries waters during the 2015 (2014/15) fishing season.
- Provide feedback to MPI on the otolith collection method change, including any impacts on the numbers of broken otoliths and potential impact on the ability to successfully prepare and age the otoliths.
- Prepare report describing collection and analysis of age data from project SEA2015-19

## Methods

Appendix 1 provides a full description of the methods used in this project. Full methods with detailed diagrams have also been presented previously in document CCSBT-ESC/0509/12, “Catch at age of Southern Bluefin Tuna in the New Zealand longline fishery, 2001-2004”, prepared for the CCSBNT SAG/SC meetings in Taipei, Taiwan 28 August to 8 September.

### *Otolith Collection*

Observers aboard New Zealand domestic vessels and foreign charter vessels routinely obtained length estimates and collected otoliths from STN. Otoliths collected during trip 4377 were stored in epidorph vials within marked envelopes, while otoliths from trip 4378 were placed directly into the envelopes. Data detailing the trip number, fish number, fish length, date of capture, area of capture and sex for each sample were obtained from NIWA. These data were supplied in electronic form, with reference to trip and fish number as the unique identifiers.

The first 100 samples from Trip 4377 and Trip 4378 were selected to compare the potential effect of not using vials to protect the otoliths. For each sample the otoliths were removed, inspected and categorised by the number of otoliths available, the number of broken otoliths, the number of samples where at least one otolith could be weighed and the number of samples where at least one otolith could be prepared. The number of otoliths in each category were summed and compared in table format.

### *Sub-sampling of otoliths*

To obtain an adequate sample for determination of catch-at-age, a minimum of 270 randomly selected otoliths were chosen from each fishing year. More samples were selected for preparation than were needed for ageing in case some otoliths were unable to be prepared or aged. Each sample was then allocated a random number and sorted. From this random sort the first 270 samples were selected for the otolith sub-sample. The length frequency distribution of the sub-sampled otoliths was compared to the length frequency distribution of the total otolith sample to ensure that the age sample was representative of the catch. If the sub-sample was not representative, then the sub-sample was reselected. Once a representative sample was selected all sub-sampled otoliths were allocated a unique Fish Ageing Services (FAS) identification number. One otolith from each pair was weighed to the nearest milligram on an electronic balance. Only undamaged otoliths were considered for weighing.

### *Preparation and ageing*

Otoliths were prepared and aged following protocols outlined in the report of the “Direct Age Estimation Workshop of the CCSBT” held 11–14 June, 2002, in Queenscliff, Australia. Opaque zones were counted along a transect starting at the primordium and running out through the ventral arm to

the otolith edge (see Figure 1). For each otolith section aged a single image was taken, the marginal increment was measured, and the otolith edge was classified as wide (w) or narrow (n).

### ***Birthdate***

A problem in assigning age from zone counts for STN is that the theoretical birthdate is January 1 yet opaque zones are thought to be formed during winter (May–October). As the sampling of the New Zealand fishing seasons occur through the middle of the year (May–August), otoliths sampled from this period may exhibit both opaque and translucent margins. Using the number of opaque zones as an estimate of age can be misleading. For example, STN that are biologically the same age can differ by 1 year depending if the opaque increment has formed on the otolith edge. To adjust for this, zone counts can be converted to age estimates by a theoretical birthdate adjustment. This can however, lead to difficulties when comparing age estimates and biological parameters from samples caught in the middle of the year to those caught at the end of the year.

To be consistent with previous approaches (Krusic-Golub 2012; Krusic-Golub 2005) the last opaque zone formed prior to the edge of the otolith was only counted when the reader could see translucent otolith material between this opaque zone and the edge. The edge type was then recorded as wide or narrow depending on the amount of translucent material on the marginal edge relative to the thickness of the previously completed translucent zone. If all otoliths are aged consistently to this protocol, then zone counts can be post-adjusted according to the requirements of the data user. In addition, it was suggested that the marginal increment of each otolith should be measured.

### ***Marginal Increment measurement***

The percentage completion of the marginal increment formation was examined by calculating the mean index of completion (C). Indices of completion were calculated using the equation:

$$C = W_n/W_{n-1}$$

Where  $W_n$  is the width of the marginal increment (the distance from the start of the last opaque zone to the marginal edge) and  $W_{n-1}$  is the width of the previously completed annulus (the distance from the start of the second most outer opaque zone to the last opaque zone).

### ***Data analysis***

The relationship between biological attributes can be used to determine whether any inconsistencies or outliers are present within the data. Age estimates were combined with fish length and otolith mass data to check for outliers.

Length frequency distributions were produced for each of the fishing seasons. A Von Bertalanffy growth curve was fitted to the combined length-at-age data using the non-linear least squares method. The growth equation was determined using the equation:

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

where  $L_{\infty}$  indicates the mean asymptotic Fork Length (cm),  $k$  represents the growth constant, and  $t_0$  is the theoretical age at length zero.

Summaries were produced for the number of fish at each age and length, and number of fish at each length for each age (age-length key), derived from Excel spread sheets. The age composition of the sampled catch was estimated using the age-length key (ALK), and then applied to the length-frequency data for each corresponding fishing year as follows:

$$A_t = \sum_x (L_x p_{tx}) \text{ where}$$

$A_t$  = the estimated number of fish of age  $t$  in the length-frequency sample,  $L_x$  = the number of fish of length  $x$  in the length-frequency sample, and  $P_{tx}$  = the proportion of aged fish of length  $x$  which were age  $t$ .

## Results

### *Otolith data*

Numbers of otoliths collected, sub-sampled and aged for each fishing year are shown in Table 1. The length frequency distribution of the otolith sub-samples selected for ageing each year was shown to be representative of the total otolith samples collected in that year (Appendix 2). The length distribution of the sampled catch is also shown in Appendix 2 for comparison, as not all trips were sampled for otoliths.

For the 200 samples investigated for the comparison of storage methods (i.e. vials within envelopes compared with envelopes only), the results of the classification process are shown in Table 2 and Table 3. Of the otoliths placed in vials prior to storing in envelopes, 62% either had one or two broken otoliths. Of the 100 samples, at least one otolith could be weighed 80% of the time and at least one otolith could be prepared 94% of the time. Of those samples placed directly into envelopes the proportion of broken otoliths was 62%. Of the 100 samples, at least one otolith could still be weighed 79% of the time and one otolith could still be prepared 98% of the time. However, the number of samples containing both of the pair of otoliths also differed. For observer 1 (vials), 64% of the envelopes contained two otoliths while for observer 2 (no vial) 98% of the envelopes contained two otoliths.

### *Age estimation and precision*

Age was estimated for 254 STN and ranged from 2 to 24 years. The age-otolith weight relationships for each sampling year are shown in Appendix 3. Several outliers were detected within these plots and the corresponding data were removed from any further analysis.

The age-length relationship is shown in Figure 3. Of the otoliths prepared, six percent could not be read. When re-read a second time, 56% of the second age estimates made by the primary reader agreed with the first estimates and 91% were within 1 year (Appendix 4). The average percent error (APE) for the primary reader was 2.96%. The APE between the primary and secondary reader for a selected subset of otoliths (n=80) was 3.39%.

Growth parameters estimated for the 2015 fishing season are presented in Table 4, along with those estimated from previous seasons for comparative purposes. The 2015 growth curve is presented and overlaid with the age-at-length data in Figure 3.

### *Proportions at age*

The ALK for the direct age estimates for 2015 are shown in Table 5, while the proportions-at-age estimated by the ALK methods are shown in Table 6 and illustrated in Figure 4. The proportions-at-age from the past three fishing seasons are also included for comparison.

The length-adjusted numbers at age for 2015 are shown in Figure 5 and the result is summarised as follows:

- Age ranged from 3 to 24 years for 2015 (2014/15) sampled otoliths
- The age composition was dominated by age classes 6–10, bimodal on 8 and 10.

- Low numbers of samples less than 6 years of age were recorded.

## Discussion and Conclusion

The results of the sub-sampled otolith comparison to determine the potential impact on otolith damage if the otoliths were not stored in vials was completed. The results indicated that the number of samples where at least one otolith from each pair could be weighed and prepared was similar regardless of whether they were stored in vials or not. The results indicated that the change of storage method does not affect the proportion of otoliths that can be successfully prepared and aged. Complicating the result was that the two sets of samples were collected by two different observers, who most likely have different levels of skill and experience in extracting tuna otoliths. For example, the number of samples containing two otoliths was much higher from observer 2 (no vials) which may indicate a superior skill in otolith extraction. When we compared just the samples where two otoliths were collected for a sample, only 2% of the samples from observer 2 contained two unbroken otoliths, whereas 32% of the samples from observer 1 (vials) were undamaged. This provides some evidence that the vials do provide additional protection to the otoliths in storage, although the number of otoliths damaged in otolith removal prior to storage is unknown.

The estimates of precision between first and second readings from the primary reader and between the primary and secondary reader were considered good. Morison et al. (1998), suggest that levels of APE less than 5% are considered acceptable for age estimation studies. The low levels of error estimated in this study suggest consistent interpretation between readings and readers.

The estimated proportions-at-age for year 2015 was similar to previous fishing seasons in that there were few samples estimated to be less than 4 years of age (CCSBT-ESC/0509/12) and the majority of the age composition was dominated by four or five age classes (Appendix 5)

Results were presented using unadjusted age (zone count=age) and with no adjustment for birthdate or edge type. The issue of edge adjustment and comparing to data from end of year catches (Indonesian, Japanese and Australian surface fishery samples) is a complex one. The estimated proportions of catch-at-age were calculated from un-adjusted zone counts. For 72% of samples aged, the marginal edge of the otolith section was classified as wide. Thought needs to be given to the effect that this classification may have on comparisons between the age composition of midyear and end-year catches if they are to be compared.

While it was suggested that no adjustment to zone count is required when estimating for proportions for catch-at-age, adjustments will be necessary if growth estimates from this study are to be compared with growth estimates from other sources i.e. end of year samples. However, because STN do not grow at a consistent rate throughout the year, if zone counts are universally adjusted to either wide or narrow (-1 or +1), there will also be a bias. Growth in juvenile STN has been shown to peak during January to May (Farley pers. comm.). Therefore, if all zone counts are adjusted for a narrow edge (as reported in IFA2004/03), the mean length for a given age class for midyear samples will be higher than for the end of year samples. This difference may incorrectly infer that STN from New Zealand grow more quickly than STN from other areas. To compare growth, a method such as randomly allocating a wide or narrow edge to each sample maybe necessary. As this was not part of the project scope this is only a recommendation and accordingly no results have been provided using this method.

The project was able to meet all the objectives of SEA2015-19. A total of 254 age estimates were provided from STN otoliths collected by observers working aboard longline vessels fishing in New Zealand waters. By applying these data to the size frequency distributions obtained from sampling the catch, proportions-at-age for the 2015 season were estimated. The continued direct ageing data provides an important tool for stock management.

## Acknowledgements

Fish Ageing Services (FAS, PO Box 396, Portarlington, Victoria, 3223 Australia) wishes to acknowledge the Ministry for Primary Industries (MPI) for providing the funding for this work under project code SEA2015-19 and the provision of otolith samples through the MPI Observer Programme. NIWA staff members David Fisher and Lynda Griggs are thanked for their assistance in the retrieval of the otolith samples and associated data and also assistance with the MPI *age* database. The author wishes to also thank MPI staff for providing editorial assistance on this report.

## References

Krusic-Golub, K. (2015). Catch-at-age of Southern Bluefin Tuna in the New Zealand long line fishery 2012–2014. Draft Final Research Report to MFISH, Fish Ageing Services Pty Ltd. (Unpublished report held by Ministry for Primary Industries, Wellington.)

Krusic-Golub, K. (2012). Catch-at-age of Southern Bluefin Tuna in the New Zealand long line fishery 2008–2011. Draft Final Research Report to MFISH, Fish Ageing Services Pty Ltd. (Unpublished report held by Ministry for Primary Industries, Wellington.)

Krusic-Golub, K. (2009). Catch-at-age of Southern Bluefin Tuna in the New Zealand long line fishery 2005–2008. (Unpublished report held by Ministry for Primary Industries, Wellington.)

Krusic-Golub, K. (2005). Catch at age of Southern Bluefin Tuna in the New Zealand longline fishery, 2001-2004. Prepared for the CCSBT SAG/SC meetings in Taipei, Taiwan 28 August to 8 September (CCSBT-ESC/0509/12).

Morison, A.K.; Robertson, S.G.; Smith, D.C. (1998). An integrated system for production fish ageing: Image analysis and quality assurance. *North American Journal of Fisheries Management* 18, 587–598.

Morton, R.; Bravington, M. (2003). Estimation of age profiles of Southern Bluefin Tuna. CCSBT-ESC/0309/32.

## Tables

**Table 1: Numbers of Southern Bluefin Tuna otoliths collected, prepared and aged from the 2014/15 fishing season.**

Year	Samples	Prepared	Aged
2014/15	1100	275	254

**Table 2: Numbers of otoliths broken and those still able to be weighed and prepared for a sub-sample of 100 samples selected from trip 4377 where the otoliths were first placed in vials before storing in envelopes. Otoliths collected by observer #1.**

Number of otoliths	Otoliths per envelope	0 broken	1 broken	2 broken	Can weigh at least 1 otolith	Can prepare at least 1 otolith
0	3	N/A	N/A	N/A	N/A	N/A
1	33	16	17	N/A	20	30
2	64	22	30	12	60	64
Total	100	38	47	12	80	94

**Table 3: Numbers of otoliths broken and those still able to be weighed and prepared for a sub-sample of 100 samples selected from trip 4378 where the otoliths were directly placed in envelopes. Otoliths collected by observer#2.**

Number of otoliths	Otoliths per envelope	0 broken	1 broken	2 broken	Can weigh at least 1 otolith	Can prepare at least 1 otolith
0	0	N/A	N/A	N/A	N/A	N/A
1	6	0	6	N/A	4	5
2	94	2	32	60	75	93
Total	100	2	38	60	79	98

**Table 4: Von Bertalanffy growth parameters estimated from each STN catch-at-age project (SEA2015/19, STN2011/01, STN2009/01, STN2007/01, STN2006/01 and IFA2003/04).**

Project	$L_{\infty}$	K	$t_0$
SEA2015-19	180.35	0.18	-2.00
STN2011/01	178.19	0.20	-1.30
STN2009/01	185.26	0.17	-2.48
STN2007/01	179.27	0.24	-0.34
STN2006/01	187.96	0.12	-5.65
IFA2003/04	183.50	0.16	-2.52

**Table 5: Age-length-key for direct age estimates 2015 – fishing season 2014/15.**

Year (2015)	2014/15																				Total	
Fork length (cm)	Age																				Total	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20+	Total
80																						
85																						
90																						
95			2	1																		3
100				1																		1
105				2																		2
110				2	3	1	1															7
115																						
120					2	4	2															8
125						9	5	1														15
130					1	5	8	2														16
135							11	6														17
140							4	9	8		1											22
145							1	10	7	2	1											21
150							2	3	12	10	9	1										37
155								1	11	12	14	5										43
160									2	2	6	8	3		1	1			1		1	25
165										3	1	2	3	2	1	1						13
170												2		1	1	2	1	3			1	11
175													2		1	1	1	1				8
180															1							2
185																				1	1	2
190																						
195																						
200																						
205																						
210																						
Total			2	6	6	19	35	32	40	29	32	18	8	3	5	5	2	5		1	6	253

**Table 6. Proportions-at-age for the three fishing seasons (2011/12 –2014/15) using the age-length-key method.**

Season	0	1	2	3	4	5	6	7	8	9	10
2012 (2011-12)				0.0076	0.0807	0.1358	0.1477	0.2517	0.1932	0.0470	0.0110
2013 (2012-13)					0.0523	0.0851	0.0929	0.1619	0.2539	0.1353	0.0388
2014 (2013-14)			0.0012	0.0136	0.0902	0.1492	0.1212	0.1230	0.1398	0.2014	0.0801
2015 (2014-15)			0.0039	0.0149	0.0158	0.0553	0.1129	0.1181	0.1604	0.1206	0.1345

Table 6 cont.

Season	11	12	13	14	15	16	17	18	19	20+
2012 (2011-12)	0.0163	0.0208	0.0185	0.0192	0.0233	0.0016	0.0101	0.0045	0.0063	0.0048
2013 (2012-13)	0.0181	0.0101	0.0193	0.0292	0.0263	0.0167	0.0176	0.0133	0.0115	0.0177
2014 (2013-14)	0.0016	0.0256	0.0049	0.0307	0.0049	0.0090	0.0015			0.0021
2015 (2014-15)	0.0722	0.0301	0.0085	0.0196	0.0203	0.0054	0.0204		0.0015	0.0190

## Figures



Figure 1: Southern Bluefin Tuna otolith section indicating the count path (black line) and the opaque zones counted (white triangles) for determining age.

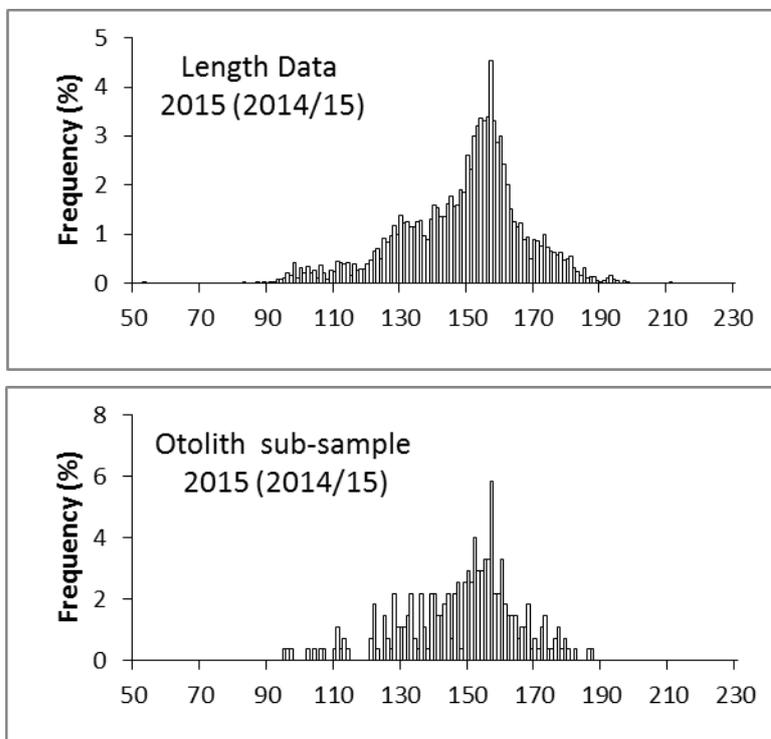


Figure 2: Length frequency distribution sampled for the 2015 fishing season from the catch and the ageing/otolith sub-sample.

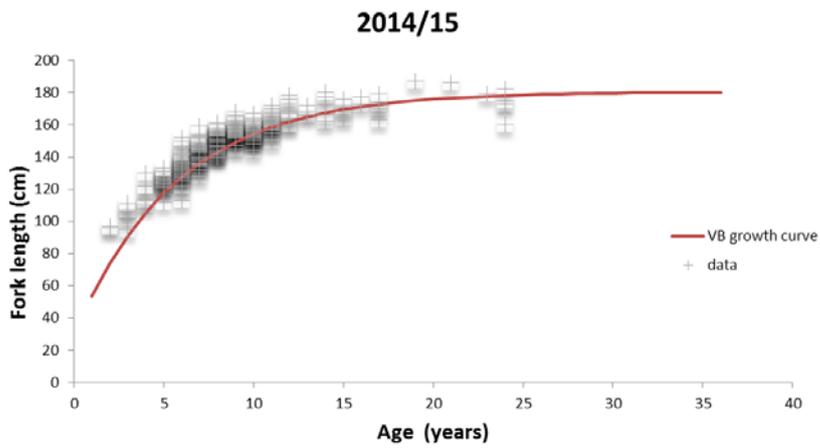


Figure 3: Length-at-age for STN for project SEA2015-19 (2015) n=254.

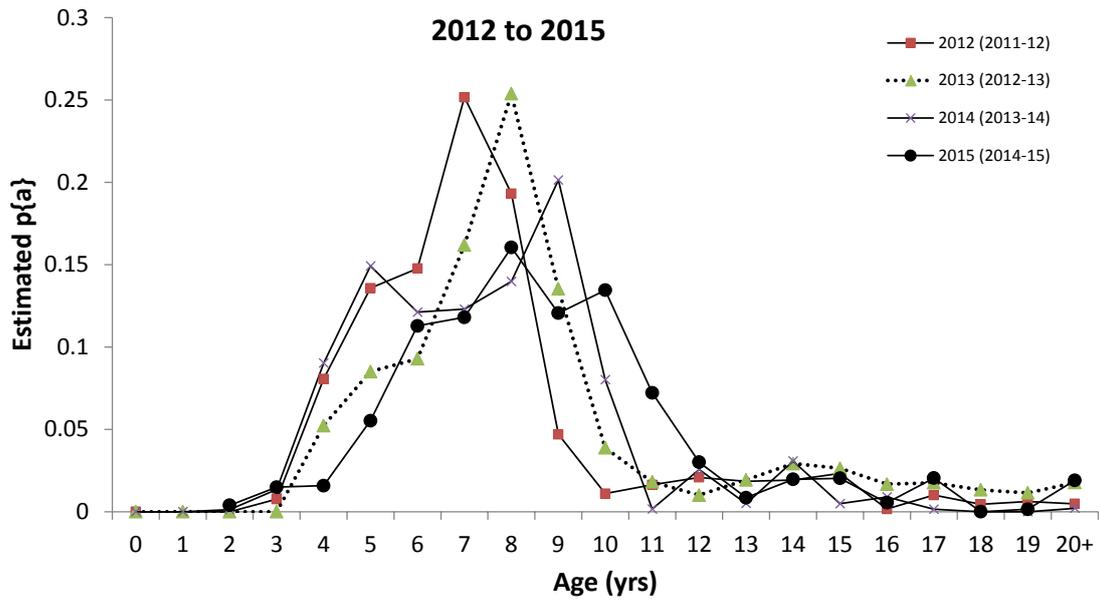
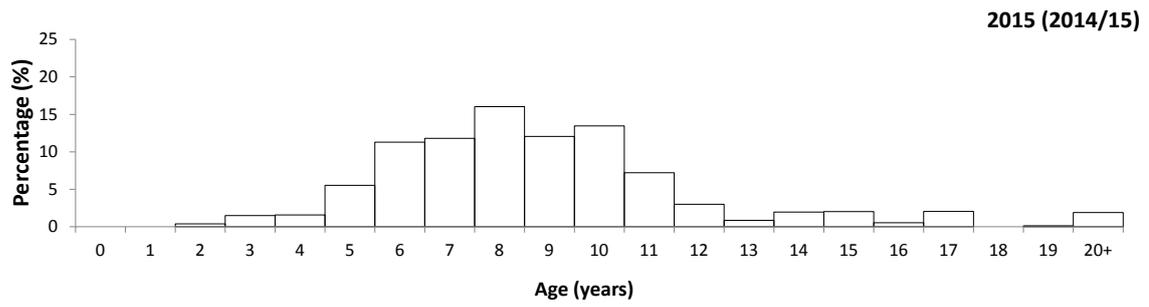


Figure 4: Proportion-of-age for the four fishing seasons, estimated using the Age-length-key method. Only the 2015 line was estimated in this study.



**Figure 5: Adjusted age composition weighted by length frequency of the catch for fishing season 2015 (2014/15)**

## Appendix 1 – Detailed Methods

### *Otolith mass*

Otolith weight is a useful diagnostic tool in assessing potential errors in age estimates and for examining patterns of otolith growth. Otolith weight has a strong relationship with fish size and age. In long-lived species, the relationship of otolith weight against estimated age may show an increased slope if the ages have been underestimated. Large variation about the relationship may indicate a lack of precision in the estimates. Also, any single outlier in the relationship may indicate incorrect assignment of age and/or incorrect length and otolith weight measurements.

All undamaged otoliths were weighed to the nearest 0.001 g on an electronic balance.

### *Preparation of otoliths*

Otoliths were prepared and aged according to standard FAS procedures and protocols. The FAS procedures are modified from Morison et al., 1998.

Otoliths were embedded, in rows of five, within blocks of clear casting resin ensuring that the primordium of each otolith was in line. Four sections, approximately 300 µm thick, were cut through their centres with a modified high speed gem-cutting saw with a 250 µm thick diamond impregnated blade. Sections from each sample were cleaned, dried and mounted on glass microscope slides (50 × 76 mm) with resin. Sections were then covered with further resin and two glass coverslips (22 × 60 mm) were placed over the top of the resin. Prepared slides were placed in an oven at 35 degrees Celsius (°C) and allowed to dry overnight.

### *Counts and measurements*

Age estimation assumes:

- Increments counted were formed on an annual basis.
- One translucent zone and subsequent opaque zone represents one annual growth increment.

Sections were examined using transmitted light under a Leitz Wild M3C binocular microscope at 25× magnification. Higher magnification was sometimes required for the examination of the fine growth increments near the otolith edge from larger, presumably older fish. Each section of the otolith was inspected, and the section with the clearest incremental structure was chosen for ageing. This was usually, but not necessarily the section closest to the primordium.

A customised image analysis system is used to measure zone distances. This system counts and measures manually marked increments and collects an image from each sample aged.

A CCD digital camera is mounted onto the dissecting microscope (Leica MZ80) and a live image is displayed on the monitor. Using the image analysis system, the positions of the opaque zones are marked with a screen cursor. The numbers of zones marked and the measurements from the first point to each subsequent mark along the transect are exported to a Microsoft database. In addition, the otolith image is automatically captured in the Joint Photographic Group (JPG) format.

To avoid the potential for biasing age estimates, all counts were initially made without knowledge of fish size, sex, location or date of capture. Once ageing of all otoliths were completed, the ageing data was combined with biological data (fish length, date of capture) and otolith weight for subsequent analyses.

### ***Image collection***

In addition to the age estimates, each otolith section was captured as an image and exported into an image database. Images were captured as a single Joint Photographic Experts Group (jpg) image.

### ***Precision of age estimates***

Repeated readings of the same otoliths provide measures of intra-reader and inter-reader variability. Repeat readings do not validate the assigned ages but provide an indication of magnitude of the error to be expected with a set of age estimates, due to variation in interpretation of an otolith. Beamish & Fournier (1981) have developed an index of average percent error (IAPE), which has become a common method for quantifying this variation. The IAPE is calculated as:

$$IAPE = \frac{100}{N} \sum_{j=1}^N \left[ \frac{1}{R} \sum_{i=1}^R \frac{|X_{ij} - X_j|}{X_j} \right]$$

where N is the number of fish aged, R is the number of times the fish are aged,  $X_{ij}$  is the  $i$ th determination for the  $j$ th fish, and  $X_j$  is the average estimated age of the  $j$ th fish. The index has the property that differences in age estimates for younger fish will contribute more to the final value than will the same absolute error for older fish (Anderson et al. 1992).

All otoliths were read twice without reference to anything other than the otolith ID and an IAPE calculated. The distribution of the differences between repeat readings was also inspected as another indicator of ageing errors, and of any bias between readings. Re-reading of the selected otoliths by the same reader/s provides a measure of precision within the readings. The purpose of the re-reading is to provide an indication of error associated with the estimates, not an agreed age.

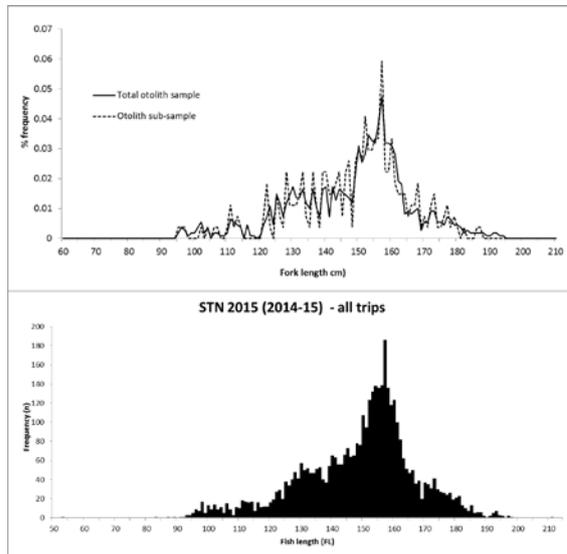
After reading each otolith twice, a final agreed age was given. If the two reads differed the otolith sample was re-read a third time and a final age assigned.

To provide a measure of inter-reader variability, for each fishing season a 10% sub-sample of otolith sections were read by a secondary reader experienced in reading STN otoliths.

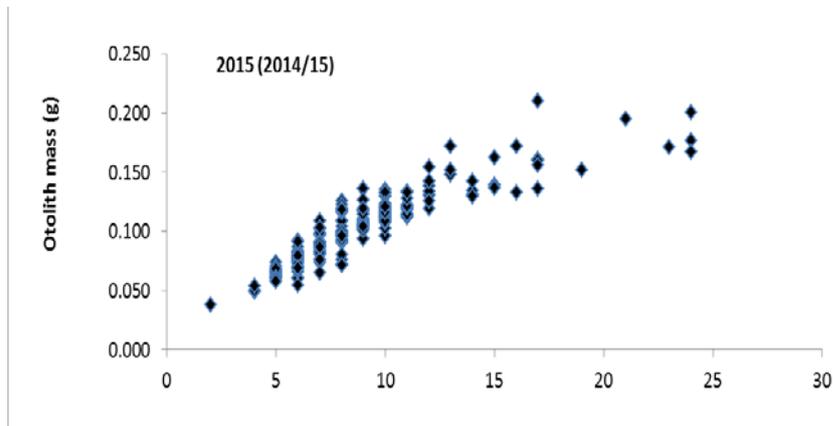
## **References**

- Morison, A.K., Robertson, S.G., and Smith, D.C. (1998). An integrated system for production fish ageing: Image analysis and quality assurance. *North American Journal of Fisheries Management* 18, 587–598.
- Anderson, J.R., Morison, A.K., and Ray, D.J. (1992). Age and growth of Murray cod, *Maccullochella peelii* (Perciforms: Percichthyid), in the lower Murray–Darling Basin, Australia, from thin-sectioned otoliths. In 'Age Determination and Growth of Fish and Other Aquatic Animals'. (Ed. D.C. Smith.) *Australian Journal of Marine and Freshwater Research* 43: 983–1013.
- Beamish, R., and Fournier, D.A. (1981). A method for comparing the precision of a set of age determinations. *Journal of the Fisheries Research Board of Canada* 36: 1395–1400.

## Appendix 2 – Comparison of length for sub-sampled otoliths and total



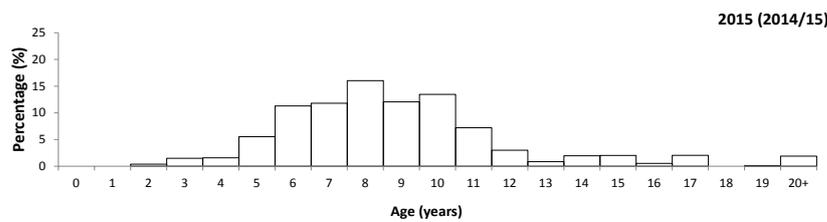
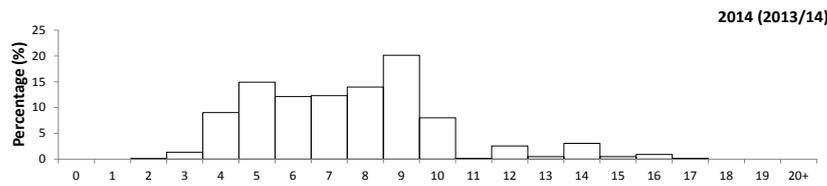
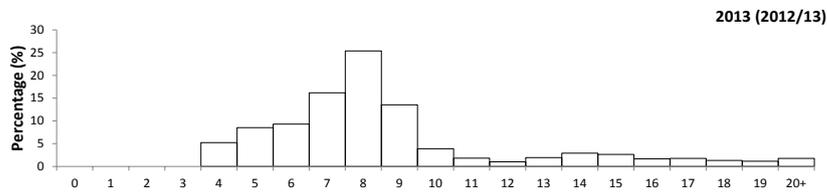
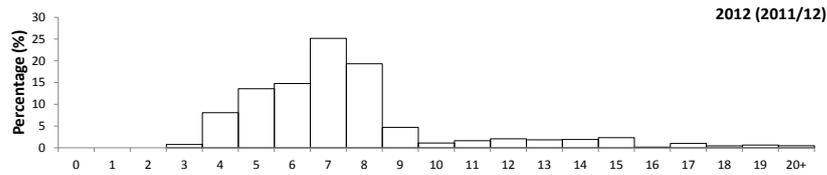
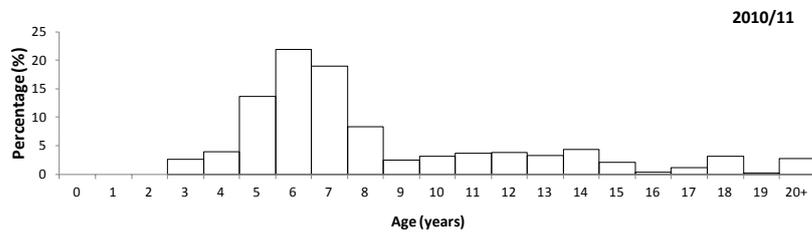
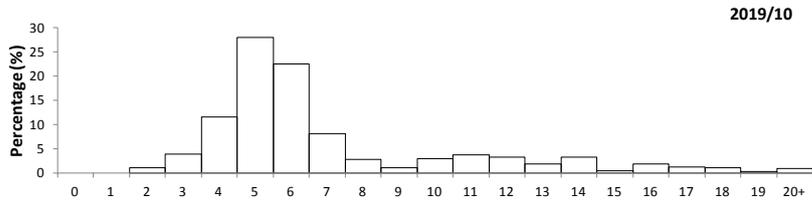
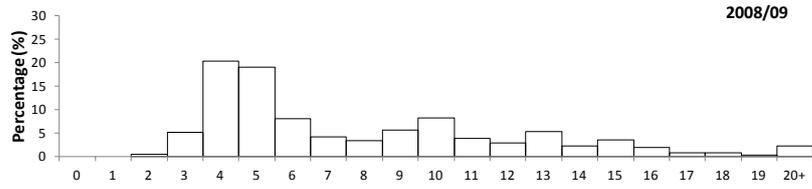
## Appendix 3 – Age-otolith weight relationships



## Appendix 4 – Age difference table (Age 1 – Age2)

Difference (Age1 - Age 2)	Age																			N	% Agree	% +/-1	
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20+				
-4								1													1		
-3																							
-2					2			2	1	2	2	2									11		
-1			1	2	3	9	8	5	4	3	1	1	1							2	40		
<b>0</b>	<b>2</b>	<b>6</b>	<b>6</b>	<b>15</b>	<b>24</b>	<b>21</b>	<b>20</b>	<b>12</b>	<b>24</b>	<b>5</b>	<b>1</b>	<b>1</b>		<b>2</b>	<b>1</b>	<b>4</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>145</b>	<b>56.42</b>	<b>90.66</b>	
1					3	9	6	10	4	5	2	2		2		1		1	3	48			
2						1		2	2	2	1	1			1				1	11			
3												1								1			
4																							
N	2	6	7	17	32	40	34	32	36	17	7	7	1	4	2	5		1	7	257			

## Appendix 5 – Adjusted age composition 2009–2015



Note: 2009 to 2011 age composition sourced from Krusic-Golub (2012) and 2012 to 2014 from Krusic-Golub (2015).