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Gene-tagging data 2019

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June, 2019
Report to the Commission for the Conservation of Southern Bluefin Tuna Operating Model and Management Procedure technical working group, Seattle June 2019.

Contents

Acknowledgments

The 2017 gene-tagging project was funded by the CCSBT, CSIRO and the EU.

2017 tagging at sea: Australian Tuna Fisheries, Marcus Stehr and crew on Yasmin.

2018 tissue collection during harvest: Seatec, Australian SBT Industry Association (ASBTIA) and members, and processing managers and staff of Australian Fishing Enterprises, Tony's Tuna International, Aussie Bites and Mori Seafood.

Diversity Arrays Technology Pty Ltd.

Fish Ageing Services

The CSIRO SBT research team and research support staff.

Abstract

Revised data from the 2016 gene-tagging pilot program and the new data from the 2017 gene-tagging program were provided to the 2019 CCSBT scientific data exchange. Details of the revision and processing of data are briefly summarised here.

The gene-tagging program has completed four years of tagging, 2016-2019, and the third harvesting season has recently commenced. The next abundance estimate in the gene-tagging time-series will be available in early 2020 for use in the 2020 stock assessment and implementation of the management procedure.

1 Introduction

The second full implementation of the gene-tagging program, which commenced in 2017, was completed in 2019 with the data exchanged to the CCSBT. This second implementation follows on from the initial pilot study in 2016 and design study in 2015. The pilot study demonstrated the logistics and feasibility of gene-tagging SBT and provided a fisheries-independent estimate of absolute abundance of 2 year old juveniles to the 2018 data exchange and Extended Scientific Committee (ESC) (Preece et al, 2018). The estimates of juvenile abundance from gene-tagging have been integrated into the SBT operating models (OMs) and are used in candidate management procedures (Anon 2016).

Gene-tagging SBT involves taking a very small tissue sample from a large number of 2-year-old SBT, releasing the fish alive, allowing 12 months for mixing with untagged SBT, and then taking tissue samples from the catch of 3-year-old fish at time of harvest. The two sets of samples are genotyped and then compared in order to find the number of fish with matching DNA; a match indicates that a tagged and released fish has been recaptured. The abundance estimate is calculated from the number of samples in the release and harvest sets and the number of matches found. The design study examined sample sizes, potential biases, costs and precision of estimates and integration of data in stock assessment and management procedure models (Preece et al, 2015).

The length range of fish included in the data analysis in 2019 has been revised, resulting in a small change to the abundance estimate from the 2016 study. A preliminary analysis of age and length data from otoliths and vertebrae has informed the revised length class.

2 Results and Discussion

A milestone report on the 2017 gene-tagging program was provided to the CCSBT in December, 2018 (Appendix A). In summary, 2017 was a very successful tagging season, with over 7500 fish tagged and released. Over 13,000 tissue samples were collected during harvest in 2018, well in excess of the target of 10,000 samples from the design study. The data and 2017 estimate of abundance of 2-year-old fish were provided to the 2019 data exchange.

The on-going monitoring program has also tagged ~8000 fish in 2018 and ~4600 in 2019. During the 2018 tagging program we noticed that there were two modes in the length frequency of the fish tagged, indicating that the length range (70-85cm, with some fish outside this range) being tagged may potentially cover more than one age class in that year. These peaks in the length frequency have not been observed in any of the other three tagging seasons.

To refine the length classes for 2 year-olds (at time of tagging) and 3 year-olds (at time of harvest, 98-109cm), we have examined fish lengths at age from direct ageing of otoliths and vertebrae. Age from otoliths and vertebrae were compared (where we had both from the same fish) to verify the vertebrae ageing method (Gunn et al, 2008). In total 80 vertebrae were aged (Figure 1). More detailed results of the ageing methods and data will be available at the ESC. Preliminary results indicate that we should exclude tagged fish outside of the length range of 75-85cm to best ensure only 2-year-olds are being included in the estimation of abundance. The harvesting length range is unchanged (98-109cm, corresponding to 3-year-olds). More vertebrae have been collected from mortalities during tagging in 2019 and during harvest sampling, to further reduce uncertainty in age of fish at time of tagging and harvest.

The restricted length range means that the number of releases used in the analysis is reduced. In both 2016 and 2017, reducing the number of fish in the release set meant that a few matches were also excluded, i.e. fish were excluded even though recaptured (matching DNA) during harvesting. The 2016 age 2 abundance estimate was revised slightly downwards. This downward revision resulted in an estimate closer to median estimate from the 2017 stock assessment models. Table 1 provides the revised 2016 and new 2017 results that were submitted to the 2019 data exchange.

There were no revisions to the methods for filtering the genotype data.

Figure 1 Age estimate from vertebrae versus fork length (cm). The grey dashed horizontal lines indicates the revised length class (75-85cm) used for tagging 2 year olds (in February/March), the lower red horizontal lines indicate the length range of fish tagged in the pilot study, and the upper red horizontal lines indicate the 98-109cm length class for 3 year-old fish during harvesting (in July-August).

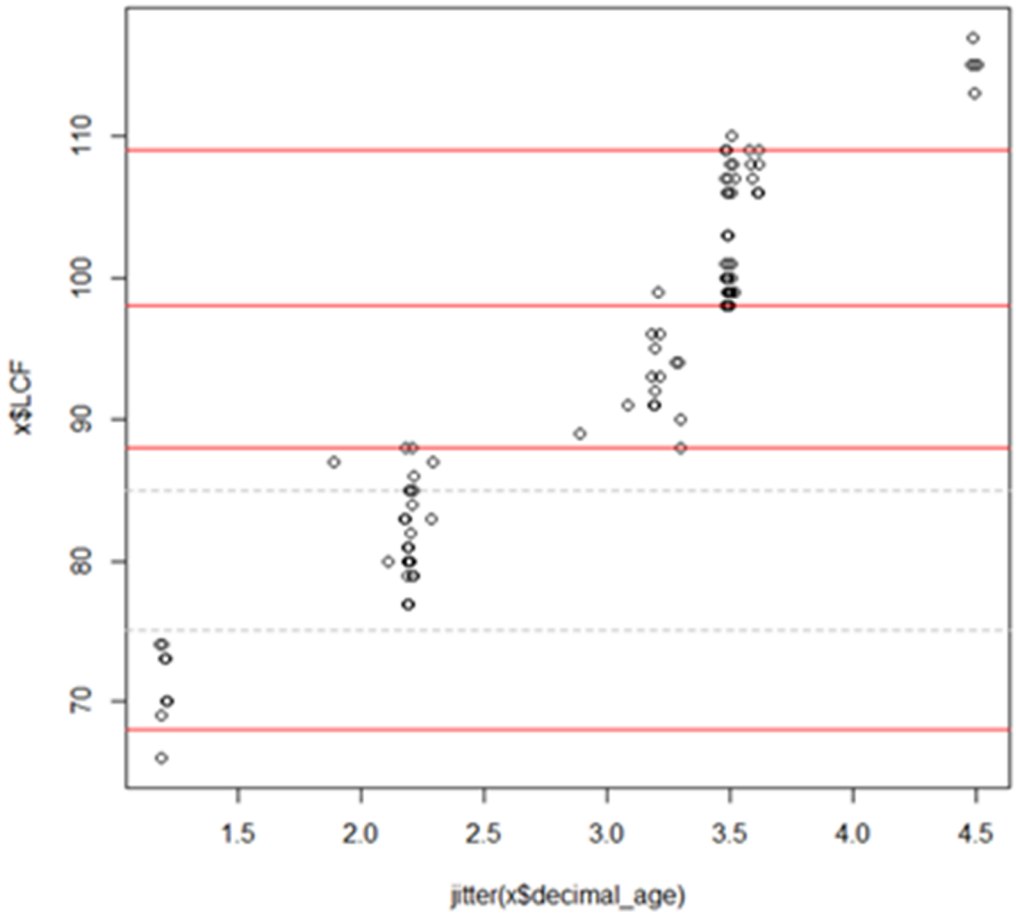

Table 1 Gene-tagging data from the 2016 and 2017 programs.

		N releases	N harvest	N matches	Abund est (millions)	CV
GT2016	All releases	3456	15390	22	2.42	0.213
	Release length 75-85cm	2952	15390	20	2.27	0.224
GT2017	All releases	7206	11932	71	1.21	0.119
	Release length 75-85cm	6480	11932	67	1.15	0.122

3 Summary

Age estimates from vertebrae were used to evaluate the length ranges being used for age 2 and age 3 fish at the time of tagging and harvest sampling, respectively. Based on this information, the length range for age 2 fish was revised to 75-85cm. Fish that were outside the refined length range were excluded from the abundance estimates provided to the data exchange this year.

References

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Appendix A

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2017 Gene-tagging Project

Milestone report – December 2018

Ann L Preece, J Paige Eveson, Peter M Grewe, Russell Bradford, Jorden Aulich and Matt Lansdell

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Diversity Arrays Technology Pty Ltd.

The CSIRO SBT research team and research support staff.

1 Background

This milestone report provides information on the second full implementation of the gene-tagging program which commenced in 2017 and is now in the final stages of completion. This second implementation follows on from the initial pilot study in 2016 and design study in 2015. The pilot study demonstrated the logistics and feasibility of gene-tagging SBT and provided a fisheries-independent estimate of absolute abundance of juveniles to the 2018 data exchange and Extended Scientific Committee (ESC) (Preece et al, 2018). The estimate of juvenile abundance from gene-tagging will be used in the SBT operating models (OMs) in 2019 and in candidate management procedures (Anon 2016).

Gene-tagging SBT involves taking a very small tissue sample from a large number of 2-year-old SBT, releasing the fish alive, allowing 12 months for mixing with untagged SBT, and then taking tissue samples from the catch of 3-year-old fish at time of harvest. The two sets of samples are genotyped and then compared in order to find the number of fish with matching DNA; a match indicates that a tagged and released fish was recaptured. The abundance estimate is calculated from the number of samples in the release and harvest sets and the number of matches found. The design study examined sample sizes, potential biases, costs and precision of estimates and integration of data in stock assessment and management procedure models (Preece et al, 2015).

The tagging component of the 2017 gene-tagging project was completed in March 2017, and the collection of tissue samples during harvest was completed in August 2018. DNA extraction and sequencing has been completed and the preliminary data analysis for calculation of an abundance estimate has commenced and will be completed in early 2019.

2 Method

The 2017 gene-tagging program followed the specifications for the pilot study as recommended in the design study. Twenty days at sea was considered the minimum viable period that would allow for bad weather and poor fishing days, based on previous experience with conventional SBT tagging projects. The design study recommended tagging and releasing 5000 fish and harvest sampling 10,000 fish.

The project involved the following steps:

1. Tag and release: Vessel charter and at-sea collection of tissue samples from age 2 fish in the Great Australian Bight during the summer of year 1 (2017).
2. Tissue collection during harvest: Collection of tissue sample from age 3 fish in year 2 (2018), during harvest of fish in farms which were caught by the Australian surface fishery.
3. DNA extraction and genotyping of tissue samples, using CSIRO SNP markers.

4. Data analysis and calculation of an abundance estimate. Provide the abundance estimate from the pilot gene-tagging program to the Extended Scientific Committee for use in candidate Management Procedure and stock assessment models in 2019.

The design study noted potential extensions to the basic design adopted, which include: tagging and resampling fish from regions outside the Great Australian Bight, tagging and resampling multiple age classes, and collection of otoliths to address uncertainties in age classes of the fish sampled.

3 Preliminary Results

3.1 Tag and release - tissue collection 2017

The 2017 tagging program was very successful, with approximately twice as many fish tagged in 2017 than in the pilot study. Over 7500 fish were tagged and released in the length range 70-85cm (see Figure 1) and 7469 samples were processed to extract DNA. Results from the tagging component of the project were reported to the Extended Scientific Committee in 2017 (Bradford and Preece, 2017). The gene-tagging tool provided sufficient DNA from the tissue biopsy, even though the sample was smaller than a grain of rice. The tool allowed for efficient sampling, with fish out of the water for around 20 seconds; quicker than conventional tagging methods, and considerably faster and less invasive than archival tagging methods.

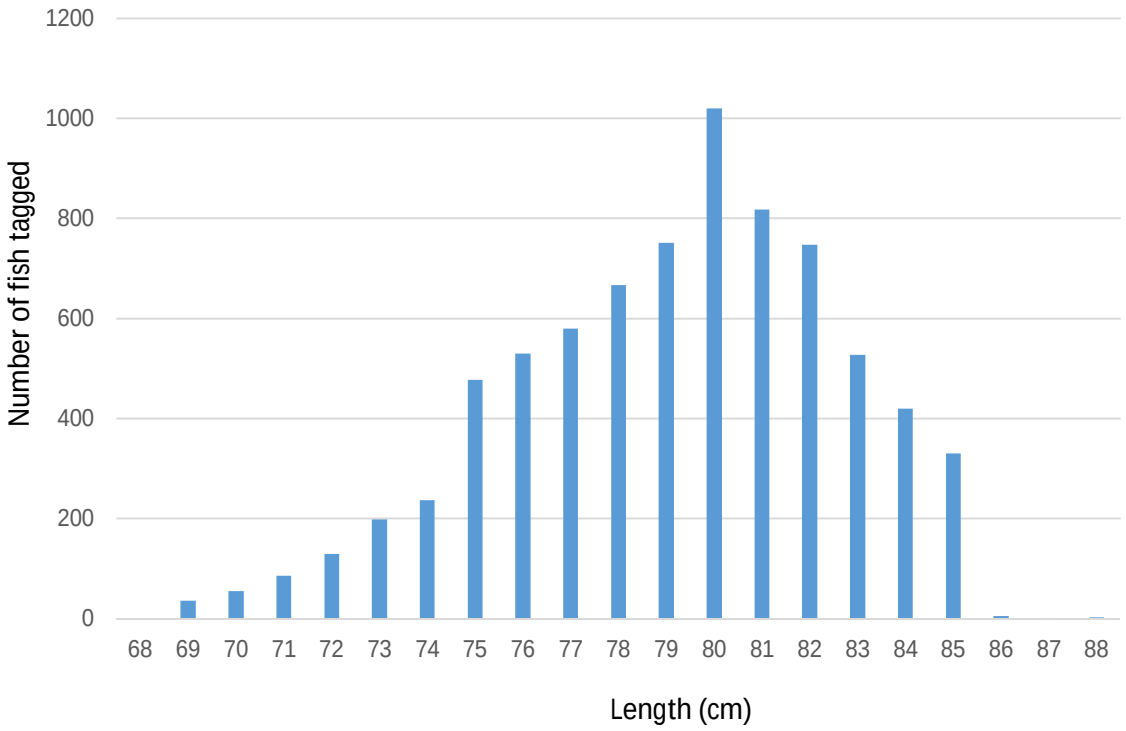

Figure 1. Length frequency diagram of southern Bluefin tuna tagged in the 2017 gene-tagging program.

3.2 Tissue collection during harvest in 2018

The method for collection of tissue samples during the commercial harvest from farms in Port Lincoln, South Australia, was developed in consultation with Industry representatives. This involves:

- Collection of tail stalks, which are removed as part of normal processing, and marking these to record length of the fish. Tails stalks were only collected from the specified size range. Date of harvest, length, collector and other details are recorded. The tail stalks were frozen and then slightly thawed at a convenient time to take the tissue biopsy.
- Collection of tissue samples from the tail stalks. Additional data was recorded (e.g. date, collector, sample number). The tissue was collected through the skin of the tail stalk using the gene-tag tool, and loaded into individually labelled vials.

All participating factories were visited during the harvesting season. Additional biological samples were also collected. We thank the managers and staff in the processing factories for allowing CSIRO and Seatec access to their facilities, and for their assistance with the project.

Over 13,000 tissue samples were collected, well in excess of the design study target of 10,000 samples, and 12,020 have been further processed to extract DNA.

Otoliths and vertebrae collected during harvest of fish with known length will be used to determine whether the length range being used for harvest sampling of 3 year olds is correct (Gunn et al., 2008). This will provide more information on impacts of uncertainties in age on the gene-tagging abundance estimates.

3.3 DNA extraction and sequencing, using CSIRO SNP markers

Nearly 20,000 tissue samples have been processed using protocols established for DNA digestion, robotic extraction and quality control. Data on processing, tracking, DNA quality and archiving were collected. As part of the quality control process Nano drop tests are used to measure the quantity and quality of DNA extracted, prior to sending the plate of extracted DNA to Diversity Arrays Technology Pty Ltd (DArT) for sequencing. Two control wells in the plate are used as a unique plate identifier, using known DNA for quality/quantity checks. An archive plate of DNA is stored at CSIRO in the -80 degree freezer. Data are recorded during all stages of the processing, to note unusual samples or results, errors or changes from original box and position to a new plate and position.

The extracted DNA is sent to DArT for sequencing using specifically designed SNP markers. Each plate holds 92 gene-tagging samples, plus control samples. Not all samples had good quality or quantity of DNA and therefore not all samples were successfully sequenced, although the success rate was very high (>96%).

3.4 Data analysis and calculation of an abundance estimate

The data returned from DArT were analysed to determine whether the same fish was in the tagged set and the harvest set (using the unique DNA fingerprint). This involved filtering the data

to exclude fish with incomplete or poor genotype information (too few SNP markers with good sequencing results).

The preliminary analysis has identified 71 matches (recaptures) in the sub-sets of releases and harvest samples that remained after filtering. A fish was determined to have been recaptured if there was a fish with a matching set of markers in both data sets (releases and harvest samples sets). This very preliminary analysis indicates that the estimate of abundance of age 2 fish in 2017 will be in the range initially considered during the design project, but substantially lower than the estimate of age 2 fish in 2016. The analysis will be further refined in 2019 to provide a final estimate of abundance.

4 Discussion

The second implementation of the gene-tagging program has successfully tagged over 7500 fish and collected samples from over 13,000 fish during the harvest. DNA has been extracted from all suitable tissue samples. Quality control filtering of the sequencing data ensures only samples with good DNA are included in the analysis. The abundance estimation stage of the analysis is still being finalised; however, 71 matches have been found in the tagging and harvest data sets. The filtering and analysis will be further refined in 2019 to provide an abundance estimate of 2-year-olds in 2017 to the CCSBT data exchange. The 2016 and 2017 abundance estimates will be used in candidate management procedures in 2019.

The CCSBT's on-going recruitment monitoring program using the gene-tagging method has had a successful third season of at-sea tagging in 2018, with over 8000 fish tagged and released. The harvest sampling stage of this project will occur in 2019. The fourth season of at-sea tagging has been funded by the Commission and will commence in February 2019. Given the unpredictable nature of fishing, we recommend that the field team collect more tissue samples when possible, as the harvest sampling can be modified to collect fewer or more samples as needed to achieve the target coefficient of variation for the abundance estimate.

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