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Australia's National
Science Agency

Report on the gene-tagging juvenile abundance monitoring program: 2016-2019

Preece AL, Eveson JP, Bradford RW, Grewe PM, Aulich J, Clear NP, Lansdell M, Cooper S, Hartog J
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Important disclaimer

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The CSIRO SBT research team and research support staff.

Contents

Abstract

The southern bluefin tuna (SBT) gene-tagging program commenced in 2016, with a pilot program that has demonstrated the technical feasibility and logistics of a genetic tagging program for SBT and its potential to provide an absolute abundance estimate for monitoring and management purposes. The program is now on-going, with four years of tagging, 2016-2019, and the third harvesting season recently completed. Estimates of juvenile (2-year-old) abundance for two years (2016 and 2017) have been provided to the CCSBT's scientific data exchange. The precision of the estimates obtained to date has been high and consistent with the expectation from the design study.

Gene-tagging SBT involves taking a very small tissue biopsy from a large number of 2-year-old SBT, releasing the fish alive, allowing 12 months for mixing with untagged SBT, and then taking a second tissue sample from a proportion of the catch of 3-year-old fish at time of harvest. The release and harvest samples are genotyped and a specific set of SNPs (genetic markers) compared in order to identify matches (i.e. recaptures). The abundance estimate is calculated from the number of samples in the release and harvest sets and the number of matches found.

The gene-tagging field team collected 4600 "release" tissue samples during 20 days at sea in 2019. The 2019 harvest sampling program has collected another ~15,000 samples (for comparison with the 2018 release samples), and DNA extraction is nearly complete. The length range used to define age 2 (release) and age 3 (harvest) fish have been reviewed through direct ageing of otoliths and vertebrae. An abundance estimate for the 2017 age-2 cohort was provided to the 2019 CCSBT scientific data exchange, and the 2016 gene-tagging pilot program abundance estimate was revised to take account of the new length range information. The next abundance estimate in the gene-tagging time-series, for the 2018 age-2 cohort, will be available in early 2020; bringing the total number of estimates to three for use in the 2020 stock assessment and implementation of the management procedure.

1 Introduction

The second full-cycle implementation of the gene-tagging program, which commenced in 2017, was completed in 2019 with data exchanged to the CCSBT. This second cycle follows the initial pilot study in 2016 and design study in 2015. The design study examined sample sizes required to achieve different levels of precision of the abundance estimates, potential sources and impacts of bias, costs and integration of data in stock assessment and management procedure models (Preece et al, 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) (Hillary et al., 2019) and are being used in candidate management procedures (Anon., 2019).

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 length range of fish included in the data analysis has been revised, based on new information on length at age (Clear et al 2019), resulting in a small change to the abundance estimate from the 2016 study. We report on progress in all stages of the gene-tagging program and the RMA request for 2020.

2 Method

The implementation of the gene-tagging program follows the recommendations of the design study (Preece et al 2015). Twenty days at sea was considered the minimum viable period that would allow contingency for bad weather and poor fishing days, based on previous experience with conventional SBT tagging projects. The recommended sample sizes were 5000 fish at release and 10,000 fish at harvest.

We report on recent progress in the following steps in the gene-tagging program:

1. Tag and release: Vessel charter and at-sea collection of tissue samples from age 2 fish in 2019.
2. Tissue collection during 2019 harvest: Collection of tissue sample from age 3 fish 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 age-2 abundance estimate for 2017 for use in candidate management procedures and stock assessment models in 2020.

We also have investigated length ranges for 2 and 3 year old fish (see Clear et al. 2019 for full details) and if there is evidence of spatial temporal patterns in fish tagged and then recaptured.

A short video on the SBT gene-tagging program was created by the CSIRO Communications group in 2019 for World Ocean Day. It is available on the CSIRO website:
https://www.csiro.au/en/Research/OandA/Areas/Marine-resources-and-industries/Fisheries/Southern-bluefin-tuna

3 Results and discussion

3.1 Tag and release

Four seasons of tagging have now been completed. In 2019, 4631 fish were tagged and released and there were 23 mortalities. The commercial fishing season did not finish until later than usual in 2019 and therefore commencement of gene-tagging was delayed. It was more difficult to find and attract fish in the required length class compared with the previous tagging seasons, and the fishing area was further east than in previous years. See trip report at Attachment A for more details. Summary of release samples sizes by year are provided in Table 1.

Table 1 Number of release samples by year.

Year	Number of Releases
2016	3768
2017	7500
2018	8000
2019	4613

3.2 Harvest sampling

The third harvest sample collection was completed in August 2019. Tissue samples were collected from fish in the length range 98-109cm, from randomly selected fish across the farms and processing factories. Over 10,000 samples are collected each year, with ~15,000 collected in 2019. Otoliths and vertebrae were also collected from fish with known length during harvest sampling to monitor the length at age relationship for the length interval for 3 year olds at harvest. We thank Seatec and the processing factories and their staff for assistance and access to fish and facilities.

All data collected from tagging and harvesting are stored in a specifically designed database.

3.3 DNA extraction and genotyping

The majority of the harvest tissue samples collected in 2019 have been processed using protocols established for DNA digestion, robotic extraction and quality control. 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 have good quality or quantity of DNA and therefore not all samples are successfully sequenced, although the success rate so far has been very high (>95%).

3.4 Investigation of length ranges

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 being targeted for tagging (70-85cm) 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 ranges for 2-year-olds (at time of tagging) and 3-year-olds (at time of harvest), we have examined fish lengths at age from direct ageing of otoliths and vertebrae (Clear et al., 2019). 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 2). Preliminary results indicate that we should only include tagged fish in the length range 75-85 cm to best ensure that only 2-year-olds are being included in the estimation of abundance. The harvesting length range is unchanged (98-109 cm, 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.

We used this revised length range in our analysis of the data from the 2017 gene-tagging program (next section). Our analysis last year of the 2016 pilot gene-tagging data used all release samples, which included fish with lengths of 68-88 cm at release. Thus, we also re-ran that analysis using the revised length range. As a result of using a reduced set of release samples, two matches were lost and the 2016 age 2 abundance estimate was revised slightly downwards (which happened to result in the revised estimate being closer to the SBT 2017 assessment estimate) (see Table 2).

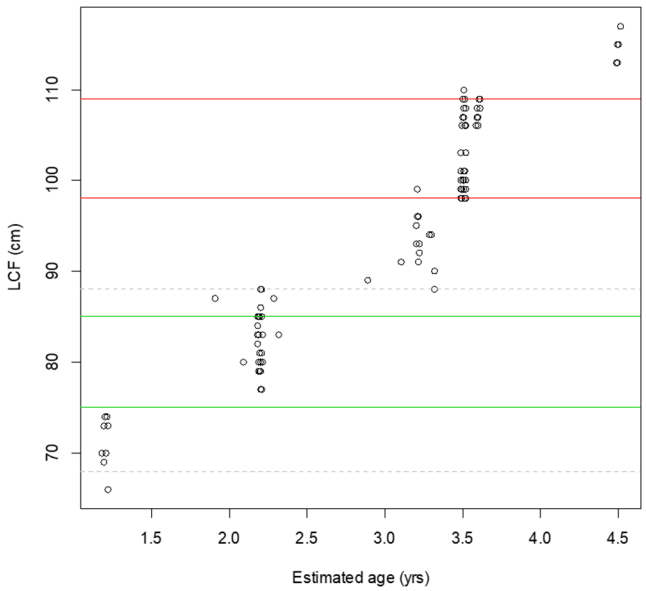

Figure 2 Vertebrae age estimate versus length. The green horizontal lines indicate the revised 75-85 cm length range used for tagging 2-year-old fish (in February/March). The range used previously was 68-88 cm as indicated by the grey dashed lines. The red horizontal lines indicate the unchanged 98-109 cm length range used for selecting 3-year-old fish during harvesting (in July-August).

3.5 Data analysis and calculation of an abundance estimate

The genotype data from the 2017 gene-tagging program were analysed to identify recaptures between the 2017 release set and the 2018 harvest set (using the unique DNA fingerprint). This involved first filtering the data to exclude samples that had: (i) significantly more heterozygous SNP markers than expected (suggesting they may be contaminated), (ii) too many SNP markers with poor sequencing information (i.e., sequence count less than 20) to reliably match with other samples, or (iii) lengths outside the specified ranges at release or harvest (see previous section). Approximately 87% of release samples and 99% of harvest samples were retained after filtering, giving 6,480 release samples (2017) and 11,932 harvest samples (2018) for analysis. Part of the reason for this high success rate is that we allow matching to be carried out using a subset of the 59 SNP markers, where a sample can be included in the analysis as long as it has at least 30 of the markers with reliable genotype calls. Of course, the two samples being compared may not have the same 30 good markers, meaning that less than 30 markers can end up being compared. Fortunately, it is usually the same markers that are poor, so out of the 6,480*11,932 = ~77.3 million pairwise comparisons, only a tiny percent use less than 30 markers, with majority of comparisons involving more than 50 markers (Figure 3). Theoretical calculations based on the observed allele frequencies for the 59 markers indicate that with 25 or more markers, the expected number of false positive matches out of 77.3 million pairwise comparisons is 0 (for truly unrelated fish and half-siblings).

The final analysis identified 67 matches (i.e. recaptures) (Figure 3). The match was perfect for 65 of these (no SNP markers being compared differed), and differed by 2 out of 52 and 2 out of 44 markers for the remaining two. This gives an abundance estimate of age 2 fish in 2017 of 1,154,020 with a CV of 0.12 (Table 2).

As the time-series of estimates of abundance of age-2 cohorts grows, we will have more information on the annual variability in recruitment. As this data series is directly monitoring abundance of recruits, it may also be used to provide an early warning of climate change impacts on recruitment.

Table 2 Gene-tagging results from the 2016 and 2017 programs giving abundance estimates of age 2 fish in 2016 and 2017 respectively. The length range to use for release samples was revised this year to only include 75-85 cm fish; thus, data from the 2016 program were re-analysed using this length range.

	Release length	N releases	N harvest	N matches	Abund est (millions)	CV
GT2016	75-85cm	2952	15390	20	2.27	0.224
GT2017	75-85cm	6480	11932	67	1.15	0.122

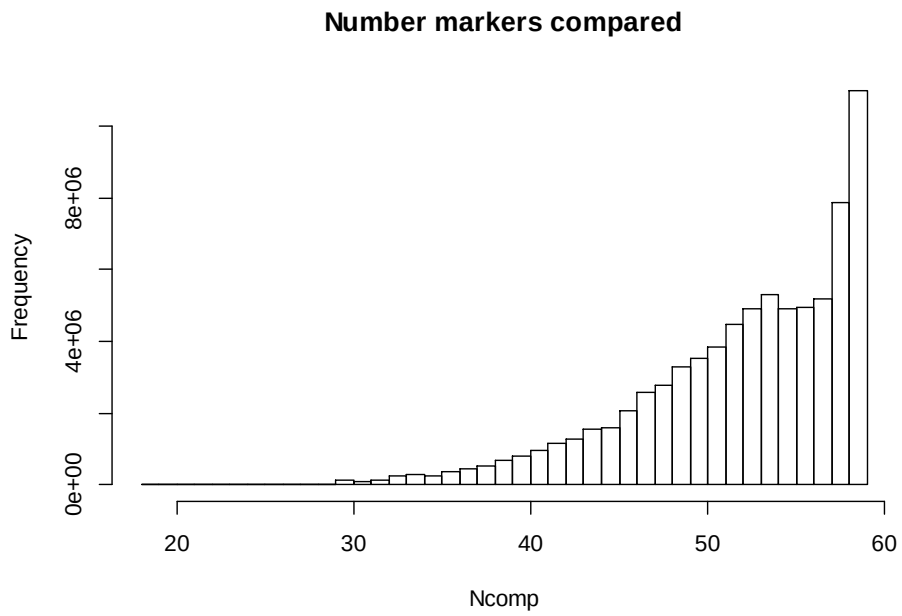

Figure 3 Number of SNP markers (out of 59) being compared between each pair of release and harvest samples (~77.3 million pairs).

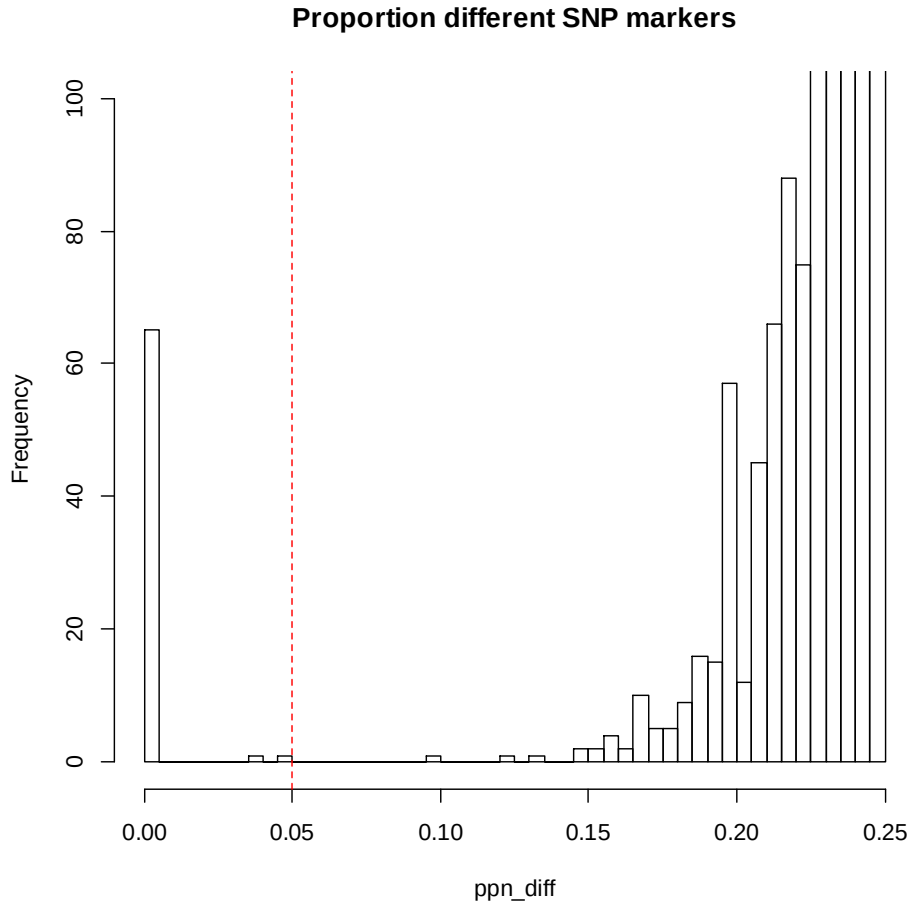

Figure 4 The proportion of SNP markers that differ between each pair of release and harvest samples (where the number being compared differs between pairs – see Fig. 2). The histogram has been right-truncated since the huge bump for the un-matched pairs (with mean ~0.50) would otherwise swamp the figure. The vertical dashed line at 0.05 indicates the cut-off allowed in the proportion of markers that differ to be considered matched samples.

3.6 Investigation of spatial and temporal patterns

Investigation into the 67 recaptures suggests there are no spatial or temporal patterns to indicate fish released in a given area, or on a given day, are more likely to have been recaptured; in fact, the distribution of the recaptures by space and time matches the distribution of releases very well (Table 3). There is also no indication that the release lengths of the 67 recaptured fish were unusual compared to the length distribution of all releases (Figure 5**Error! Reference source not found.**). If, for example, none of the smaller releases had been recaptured, we might suspect that they were actually 1-yr-olds, and thus not available in the harvest sample the next year for recapture (as the harvest sample is focussed on 3-yr-olds) – but this was not the case. For the harvest lengths, there is a slightly higher proportion of recaptures in the 99-cm length bin compared to the distribution of all harvest samples (Figure 5), but given there is no trend, there is no reason to suspect anything except random variability. If there had been a trend, such that the proportion of harvest samples that were recaptured decreased with length, then we might suspect that many of the larger harvest fish were actually 4-yr-olds, so that a match with fish released as 2-yr-olds the year before would not be possible – but, again, this was not the case.

Table 3 Number and percent of releases and recaptures by (a) release date and (b) release location.
(a)

Release Date	No. Releases	% Releases	No. Recaptures	% Recaptures
15/02/2017	1094	14.6%	11	16.4%
16/02/2017	748	10.0%	7	10.4%
17/02/2017	712	9.5%	2	3.0%
19/02/2017	379	5.1%	6	9.0%
20/02/2017	1149	15.4%	10	14.9%
21/02/2017	553	7.4%	2	3.0%
24/02/2017	823	11.0%	6	9.0%
25/02/2017	184	2.5%	3	4.5%
26/02/2017	270	3.6%	2	3.0%
27/02/2017	818	11.0%	10	14.9%
1/03/2017	47	0.6%	0	0.0%
4/03/2017	174	2.3%	3	4.5%
5/03/2017	518	6.9%	5	7.5%
Total	7469	100.0%	67	100.0%

(b)

Release Location	No. Releases	% Releases	No. Recaptures	% Recaptures
133, -32.3	2020	27.0%	18	26.9%
133.2, -32.6	189	2.5%	1	1.5%
133.3, -32.6	1149	15.4%	10	14.9%
134, -33.7	886	11.9%	5	7.5%
134.7, -34.8	3178	42.5%	33	49.3%
136.6, -35.3	47	0.6%	0	0.0%
Total	7469	100.0%	67	100.0%

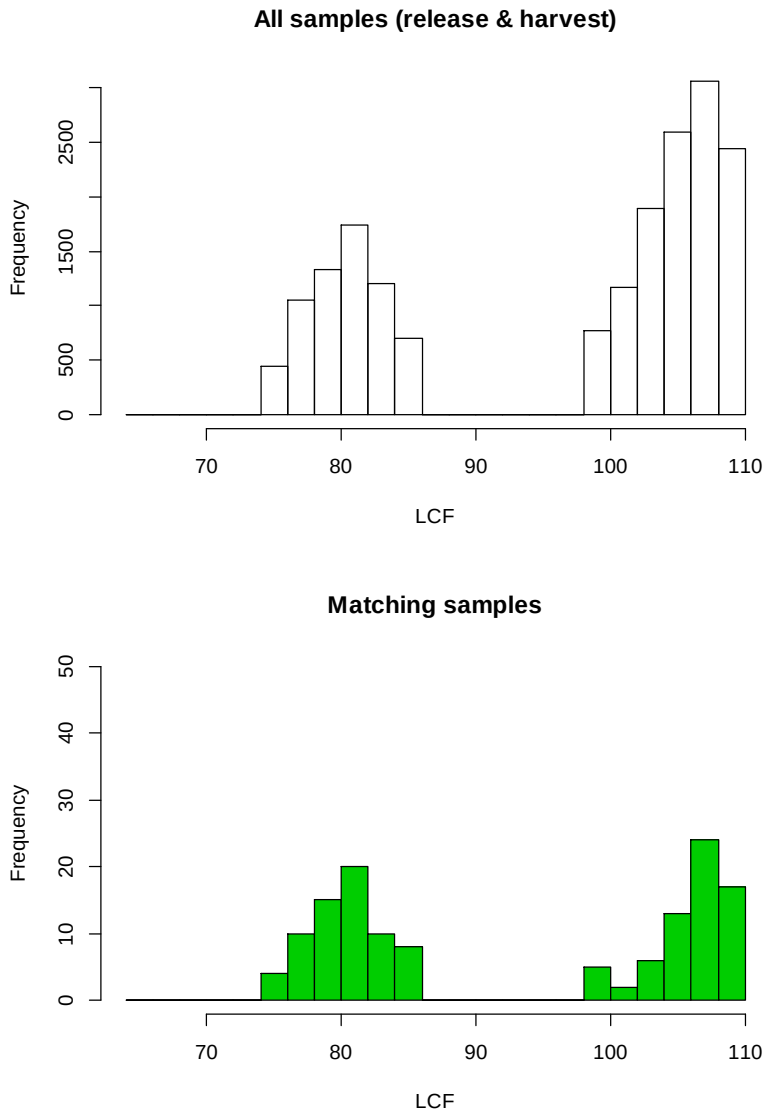

Figure 5 Comparison of length distributions for all release and harvest samples compared to those for just recaptured fish.

3.7 Potential sources of bias and uncertainty that were considered in the gene-tagging design study

The gene-tagging design study examined: potential sources of bias and uncertainty, and whether the gene-tagging program could resolve them; mitigation measures to avoid potential biases; and potential extensions to the gene-tagging program that could be considered in the future. These issues are very briefly summarised in Table 4 below. They are discussed more thoroughly in Preece et al., 2015. An overdispersion factor to encompass some of the additional uncertainty is included in the SBT operating models, but will require a longer time-series of gene-tagging data before it can be estimated.

Table 4 Summary of potential sources of bias and uncertainty

Potential source	Evidence	Impact	Mitigation
Spatial heterogeneity. Hypothesis that a percent of the juvenile population never come to the GAB	Basson et al., 2012 concluded: unlikely there is a population residing off South Africa (or elsewhere). Total catches of juveniles in South Africa are very small. No support for this hypothesis from the CKMR model or SBT OM using the HSP and POP data.	Population abundance estimate would be for Great Australian Bight (GAB) fish only	Investigate 'q' factor in models; Further sampling older ages to test hypothesis
Incomplete mixing	Different behaviour of age 1 fish tagged in the 2000s in WA. No evidence of non-mixing for ages 2 & 3 from previous tagging.	Polacheck et al. 2006; Basson et al. 2012: suggest mixing is good. Same impact as above.	Consider research projects to address juvenile spatial dynamics
Tagging mortality	Hoyle et al 2014, identified conditions leading to high tagging mortality rates for tropical tunas. Initial trials of GT tools demonstrated no mortality (Bradford et al, 2015). SBT robust to more invasive archival tagging.	Assumed low (zero) for this type of tagging on SBT. Impact of high tagging mortality would be over-estimates of abundance.	Good tagging protocols developed. Damaged or injured fish are not tagged. GT method is rapid with fish out of water 10-20 seconds only.
Ageing uncertainty	Potential for annual variation at length at age. GT results indicate fish in release length range are equally represented in recaptures.	Assumed low – given direct age data. Impact would be over-estimate of population size, or increase in variance.	Direct age data used to examine and refine length classes. Ability to correct for this in the models.
School fidelity	No evidence from conventional tagging studies - with 12 months for mixing	Would increase variance but not bias	Tagging protocols developed – frequent move to new schools

4 RMA request for 2020

For the 2020 tagging component of the gene-tagging program we are requesting permission for a 2t Research Mortality Allowance (RMA). In previous years we have requested 3t RMA, however the careful landing by the crew of Yasmin when poling fish on board for tagging has resulted in fewer mortalities each year (<~0.5t). An allowance of 2t will provide a buffer in case of unexpected conditions that result in an increase in mortalities (e.g. hungry or more aggressive fish may be at more risk of damage from the lure during poling operations, difficult sea conditions).

Biological samples, i.e., otolith, vertebrae, and gonads, are collected from the mortalities.

5 Summary

The on-going gene-tagging program has completed the collection of samples, genotyping and identification of recaptures to provide an estimate of abundance of the age 2 cohort in 2017.

The estimate of abundance from the 2016 pilot program (age 2 cohort in 2016) was revised given new information on the length range of age 2 fish, where the new length at age information came from direct ageing of vertebrae, verified against otoliths (see Clear et al 2019).

The next abundance estimate, from the 2018 tagging and 2019 harvest sampling, will be available in early 2020 for use in the 2020 stock assessment and the new management procedure.

The 2019 tagging was successful, although the 5000 fish target was not quite reached. This can be compensated for during the harvest sampling in 2020 when additional numbers of fish can be sampled to maintain a high CV of the estimate.

There are no spatial or temporal patterns in the 2017 gene-tagging program data (2017 tagging, 2018 harvest sampling) to indicate fish released in a given area, or on a given day, are more likely to have been recaptured. There is also no indication that the release lengths of the 67 recaptured fish were unusual compared to the length distribution of all releases, and there was no trend in the recapture lengths. This indicates that the target length ranges used for tagging and harvest sampling are appropriate.

In February-March 2020 the gene-tagging field team aim to tag another 5000 juvenile SBT, for which 2t of RMA is requested, however only a small number of mortalities is expected based on previous years' work.

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Attachment A – Gene Tagging 2019 Fieldwork Report. Russ Bradford

The 2019 CSIRO/CCSBT gene tagging fieldwork was undertaken from 13 March through 07 April 2019. The trip consisted of two legs, each of approx. 10 days. The commercial surface fishery was extended this year as a result of large numbers of small SBT, mixed-size schools, and unusual water conditions resulting in no clear aggregations of fishable schools. This, in turn, delayed the start of the gene tagging program by approximately 2 weeks.

Fishing effort was distributed differently to previous years, with fishing extending much further to the east (Figure A.1). Fishing was bounded by 134.36E / 34.40S in the northwest and 138.58E / 36.41S in the southeast.

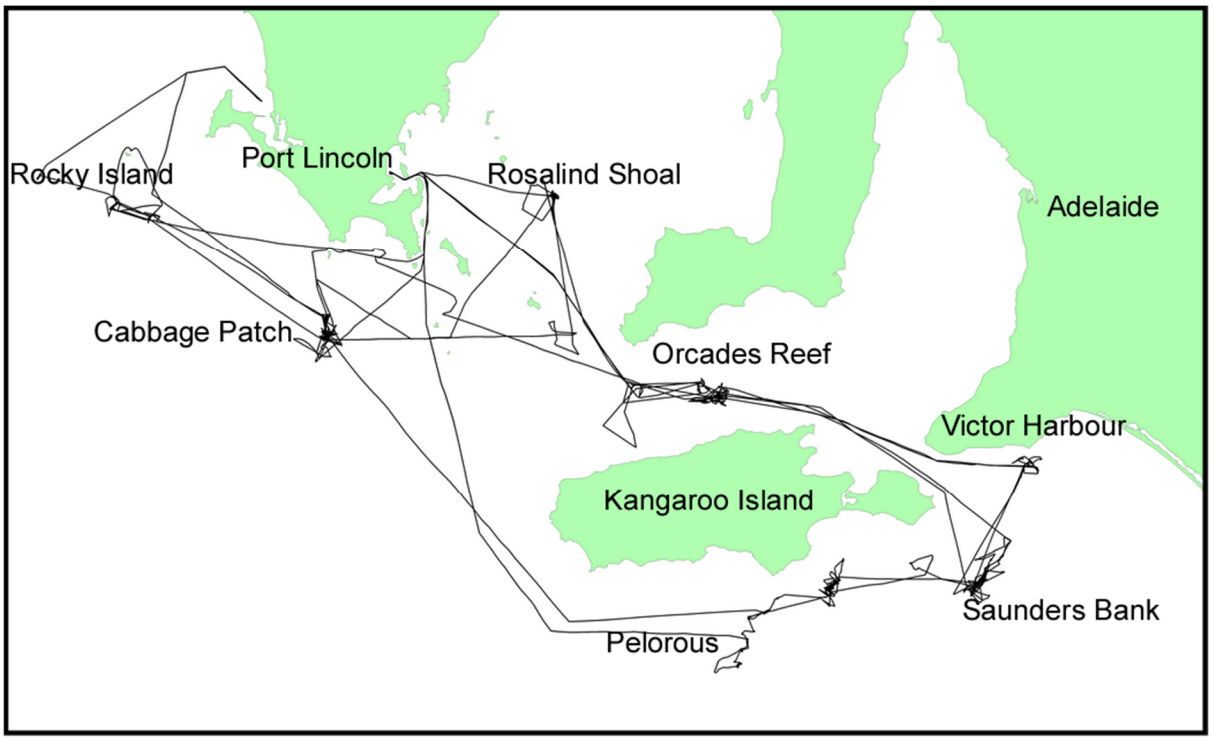

Figure A.1. Track of the FV *Yasmin* in 2019 showing major regions of gene tagging activity.

Tagging effort was spread more broadly than in 2018 (Table A.1). Key regions for tagging included the Cabbage Patch to the south of Port Lincoln, Orcades Reef to the north of Kangaroo Island, and Pelorous Islet to the south of Kangaroo Island. In general, fish to the east were larger and appeared more well fed than fish to the west. The industry observed large amounts of bait in the eastern region, and this may have been one cause of the reluctance of SBT to follow the tagging vessel.

In total, 4631 Southern Bluefin Tuna were caught and tagged; 1169 were caught by pole and line but not tagged, 87 were caught on the troll line, and 23 SBT were killed (biological material retained, see Table A.2). Over the course of the 2019 field work 17 Yellowtail Kingfish (*Seriola lalandi*) and three Trevally (*Pseudocaranx georgianus*) were caught as bycatch. One conventional gamefish-tagged fish was poled during the course of this work.

The target fork length in 2019 was reduced to include SBT between 75 and 85 cm (FL). The fork length of SBT tagged peaked at 80 cm (Figure A.2), however, the distribution of lengths was slightly biased to larger fish (1169 <80 cm FL cf 2695 >80 cm FL).

Table A.1. Number of Southern Bluefin Tuna tagged and released by region fished.

Region	Number SBT Tagged
Allthorpe Islands	256
Cabbage Patch	1474
Orcades Reef	1148
Pelorous Islet	924
Rocky Island	573
Rosalind Shoal	25
Saunders Bank	72
Victor Harbour	159

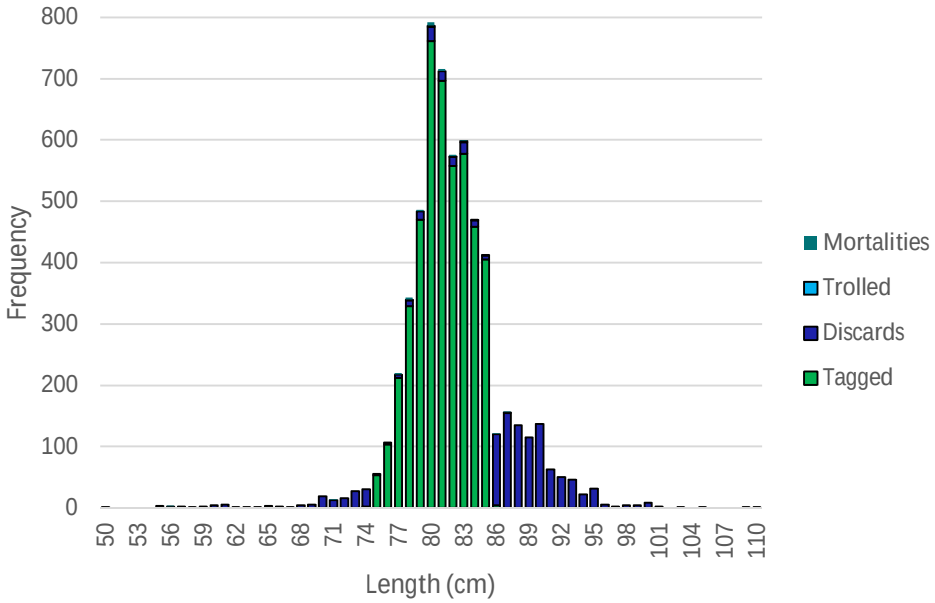

Figure A.2. Length frequency of all southern Bluefin tuna caught during the 2019 gene tagging fieldwork.

Fewer SBT were caught on the troll lines in 2019 compared to 2018: 87 and 111, respectively. As a general observation the further east fishing was happening the lower the incidence of troll-caught SBT (no other species were caught on the troll lines) despite similar levels of trolling effort. Again, this may reflect the general well-fed appearance of fish in the east compared to the west, and their reluctance to surface and take a bait. The majority of the troll-caught SBT were in the 75 to 90 cm FL range. Figure A.3 provides the weight-length relationship for all troll-caught SBT.

Mortalities arising from the fishing (pole and troll-caught) were lower than in 2018: 23 and 39, respectively. In addition to fewer SBT being caught by troll line, fewer troll-caught SBT were killed as a result of the trolling operations. Very few SBT were caught that were outside of the target size range for gene tagging. However, in general, the fork length of troll-caught fish decreased from east to west. The main cause of death was attributed to gill bleeds from poling operations. Mortality rates increased as tagging rates increased, but were also affected by the activity level of the SBT. Fish which were hungrier or more aggressive towards the lure were more likely to be foul-hooked in the gills. Figure A.4 provides the weight-length relationship for SBT killed during the course of 2019 field work.

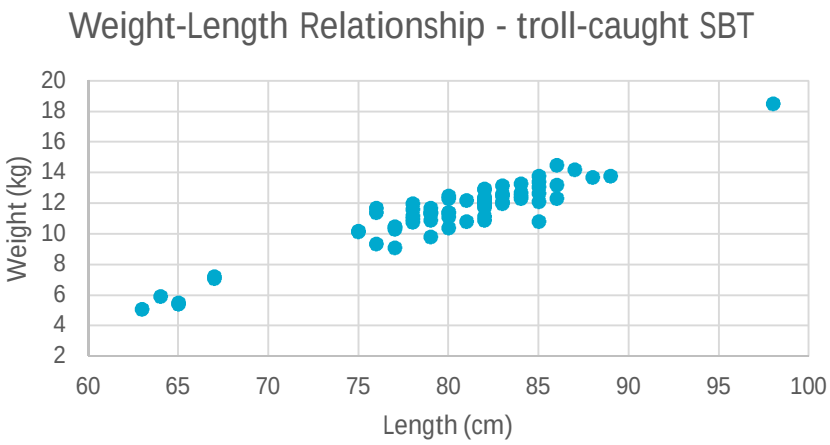

Figure A.3. Weight-length relationship of troll-caught southern Bluefin tuna.

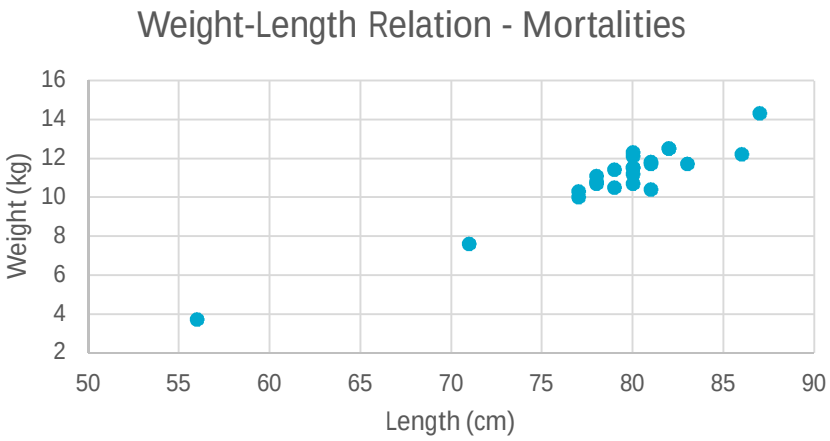

Figure A.4. Weight-length relationship of Southern Bluefin Tuna mortalities.

Several SBT were recaptured throughout the tagging operations. Suspected re-tagged fish are noted in the data sheets and in the database (comments field). Figure A.5 shows a recaptured SBT with a previous tag wound. This fish was presumed to have been tagged a week prior in the same region. On the second leg, in the same region, another suspected recapture occurred. The previous wound had healed well and was represented by a slight discolouration of the scales from where the gene tag sample had been taken. Unfortunately no images of this fish are available.

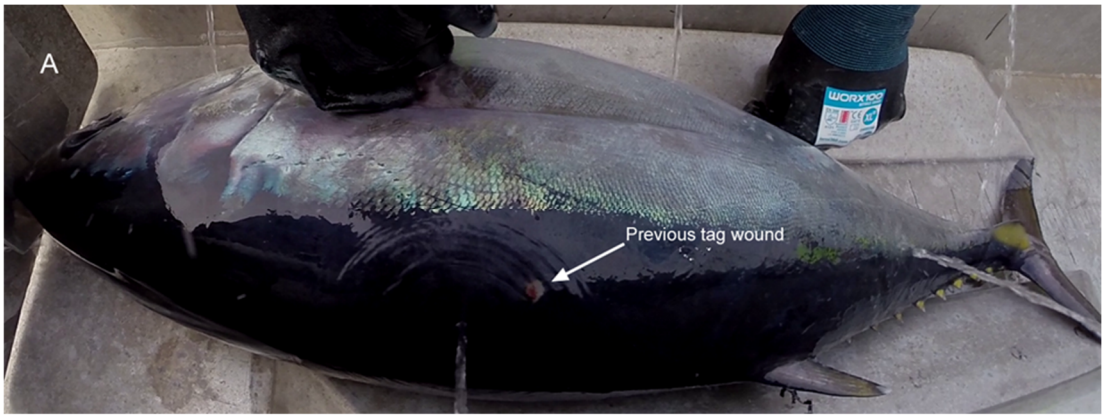

Figure A.5. Recaptured SBT, prior to taking the second gene tag sample.

Table A.2. Southern Bluefin Tuna killed for the collection of biological materials. These fish were deemed at the time of capture to be unlikely to recover from the capture process and survive a return to the water.

Date	Cause	Length (cm)	Weight (kg)	# Otoliths	Heart	Muscle	Tail_Stock
14/03/2019	Poling Gill Bleed	82	12.5	2	Y	Y	Y
14/03/2019	Poling Gill Bleed	80	10.7	1	Y	Y	Y
14/03/2019	Poling Gill Bleed	80	11.2	2	Y	Y	Y
14/03/2019	Poling Gill Bleed	82	12.5	1	Y	Y	Y
15/03/2019	Poling Gill Bleed	78	11.1	2	Y	Y	Y
16/03/2019	Trolling Death	56	3.7	1	Y	Y	Y
16/03/2019	Poling Gill Bleed	80	12.3	2	Y	Y	Y
16/03/2019	Poling Gill Bleed	71	7.6	2	Y	Y	Y
18/03/2019	Poling Tail Break	81	11.7	2	Y	Y	Y
18/03/2019	Poling Gill Bleed	81	10.4	2	N	Y	Y
20/03/2019	Trolling gill bleed	79	11.4	2	Y	Y	Y
20/03/2019	Poling Gill Bleed	77	10.3	2*	N	Y	Y
20/03/2019	Poling Gill Bleed	77	10	2	N	Y	Y
20/03/2019	Poling Gill Bleed	78	10.7	2	Y	Y	Y
20/03/2019	Poling Gill Bleed	79	10.5	2	Y	Y	Y
20/03/2019	Poling Gill Bleed	83	11.7	1	Y	Y	Y
22/03/2019	Poling Gill Bleed	87	14.3	2	N	Y	Y
22/03/2019	Trolling gill bleed	78	10.8	2*	Y	Y	Y
28/03/2019	Poling broken tail	81	11.8	2*	Y	Y	Y
4/04/2019	Trolling gill bleed	80	11.5	2	Y	Y	Y
6/04/2019	Poling Gill Bleed	80	11.5	2	Y	Y	Y
6/04/2019	Poling Gill Bleed	86	12.2	2	Y	Y	Y
6/04/2019	Poling Gill Bleed	80	12.1	2	Y	Y	Y

* Both otoliths recovered, but only one intact.

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Contact us

1300 363 400
+61 3 9545 2176
csiroenquiries@csiro.au
www.csiro.au

For further information

Oceans and Atmosphere
Ann Preece
+61 3 6232 5222
ann.preece@csiro.au
www.csiro.au/en/Research/OandA