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Update on the SBT gene-tagging recruitment monitoring program 2025

Preece AL, Eveson JP, Hartog JR, Dell J, Aulich J, Potter N, Cooper S, Grewe P.

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Diversity Arrays Technology Pty Ltd.

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1 Abstract

The CCSBT Southern Bluefin Tuna (SBT) gene-tagging program provides an estimate of the absolute abundance of the age-2 cohort, for use in the Cape Town Procedure and stock assessment models. The program has been running since 2016. Due to delays in sequencing last year, we were unable to provide an estimate of age-2 abundance in 2022 to the CCSBT ESC29. Thus, two new estimates of abundance, for the 2022 age-2 cohort and 2023 age-2 cohort, are available this year, and we provide an update on tissue sample collections in 2024 and 2025.

The CCSBT has reduced the budget for the gene-tagging program in 2025 and 2026. The reduced program involves fewer days at sea in 2025 and collection of a limited number of samples in 2025 and 2026. In 2026 the at-sea tagging component of the program will not be conducted and therefore monitoring of recruitment of the 2026 age-2 cohort will not occur.

To estimate abundance of the 2022 age-2 cohort, muscle tissue samples were collected from over 5000 age-2 fish, in 2022, and the fish were released alive. In 2023, over 15,000 harvest samples were collected from age-3 fish. Sampling logistics were more complex than in previous years with tissue samples collected during offshore processing. Samples from a wider length range were collected, to allow for a potential shift in length at age. DNA extraction was completed, but genotyping was delayed, hence the abundance estimate was not available for the ESC last year. Over 78 million comparisons were made and 38 matches detected, giving an estimate of age-2 abundance in 2022 of 1.969 million (CV = 0.162).

To estimate the abundance of the 2023 age-2 cohort, 3000 fish were tagged and released in 2023 (this is below the target sample size). During the 2024 commercial harvest, over 15,000 samples were collected, again from a broader length range to allow for observed shifts in length at age in recent years. These samples have been processed, and genotypes compared to detect matches. Over 36 million comparisons were made and 14 matches detected, giving an estimate of age-2 abundance in 2023 of 2.62 million (CV = 0.267, which is above the target CV of 0.25 due to the small number of matches).

The 2022 and 2023 results have been provided to the 2025 CCSBT data exchange and will be used in the Cape Town Procedure to recommend the TAC for 2027–2029.

The tagging field work in 2024 occurred over 20 days in March. Over 3700 fish were tagged and released but this is below the 5000 fish target. Harvest sampling of age-3 fish in 2025 is underway. The abundance estimate for this cohort will be available in 2026 for the next assessment of stock status.

At-sea tagging field work in 2025 was conducted under the reduced gene-tagging budget. Sea days were reduced to 12 days, and over 3600 fish were tagged, which is again under the target number. Harvest sampling of age-3 fish will occur in 2026, and the abundance estimate will be available in 2027.

We are not seeking Research Mortality Allowance in 2026 because the gene-tagging field work will not be conducted.

2 Introduction

The gene-tagging program provides an annual estimate of absolute abundance of juvenile Southern Bluefin Tuna (SBT). These estimates of abundance are used in the Cape Town Procedure (Anon., 2020) to provide a management recommendation on the global Total Allowable Catch (TAC), in the annual review of indicators, and in the assessment of stock status (e.g., Hillary et al., 2023).

Gene-tagging SBT involves “tagging” fish by taking a very small muscle 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 muscle tissue samples from the catch of 3-year-old fish at time of harvest. The tissue samples from tagging and harvesting are processed to extract DNA which is then genotyped, and the genotypes from the two sets of samples are compared to detect samples with matching DNA. A match indicates that a tagged and released fish has been recaptured. The estimate of abundance is calculated from the number of samples in the release and harvest sets and the number of matches found.

The gene-tagging program follows the specifications for the pilot study as recommended in the design study (Preece et al., 2015). The design study examined sample sizes, potential sources of bias, costs and precision of estimates and integration of data in stock assessment and management procedure models. Twenty days at sea was considered the minimum viable period to achieve the desired samples size, allowing for bad weather and poor fishing days, based on previous experience with conventional SBT tagging projects. The design study recommended tagging and releasing 5,000 fish and harvest sampling 10,000 fish.

This report covers the data collected for calculation of the estimate of abundance of age-2 fish in 2022 (which was not available for last year’s report due to delays in sequencing) and 2023 (as per schedule). We also provide an update on the gene-tagging program in 2024 and 2025. The estimate of abundance, for the age-2 cohort in 2024, will be available in early 2026.

The CCSBT agreed to a reduced gene-tagging program in 2025 and 2026 because of budget constraints. The reduced program involves fewer days at sea in 2025 and collection of limited numbers of samples in 2025 and 2026. In 2026 the at-sea tagging component of the program will not be conducted and therefore monitoring of recruitment of the 2026 age-2 cohort will not occur.

3 Data collection for an estimate of abundance of age-2 cohort in 2022

3.1 Tagging in 2022

In 2022, the gene-tagging field team collected over 5,000 tissue samples from age-2 fish and released these tagged fish alive, to mix with the untagged population. The tagging field trip occurred over 20 days, from a chartered vessel and pole and line fishing crew. A tightly specified length range was used (75–85 cm fork length (FL)), and only fish in excellent condition were sampled. This was the 7th gene-tagging field trip (noting 2020 was cancelled mid-trip due to COVID-19 and poor fishing).

3.2 Harvest sampling in 2023

In 2023, tissue samples were collected from 15,000 age-3 fish as they were processed during the harvest from the purse seine fishery farms in Port Lincoln, South Australia (June-August). This is over the 10,000 fish target level of sampling. The method for collection of tissue samples during the commercial harvest was developed in consultation with Industry representatives. In 2023, this involved sampling from both onshore and offshore processing. Collection of tissue samples is from the tail stalks. Tissue is collected through the skin using the gene-tag tool (Bradford et al, 2015) and loaded into individually labelled vials. Additional data were recorded (e.g., date, collector, sample number).

The harvest sampling is designed to select fish at random from across the different farms and processing factories, and throughout the full harvest period. A wider range of length classes were sampled to cover changes in length at age observed over recent years.

3.3 2022 cohort results

All 20,000 muscle tissue samples (release 2022 and harvest 2023) were processed for DNA extraction and quality control. Samples are tracked throughout lab processing, archiving, and DNA quality control. Data were recorded during all stages of the processing, to note unusual samples or results, errors, or changes from original plate and well position, to a new plate and/or well position.

A concern with the 2022 gene-tagging program was the apparent decrease in the size of fish available during tagging relative to the target size range (Figure 1, i.e. only upper range of the age 2 length distribution has been sampled). To address this, we collected tissue samples at harvest from a broader range of sizes (89–109 cm FL) than planned in the original design study, to ensure sampling the age 3 cohort if they were growing slower. Simulation analysis indicates that we can use all the samples to calculate the abundance estimate. This is because of the combination of 1) growth rates being quite variable i.e. larger age-2 fish are not always larger at age-3 (also noted in previous years' data), and 2) although we sampled the upper range of the age 2 length distribution, we sampled most of the age 3 distribution, as shown by the distribution of matches (Figure 1). If we may have missed some of the smaller age 3 fish, the abundance estimate would be very slightly biased low.

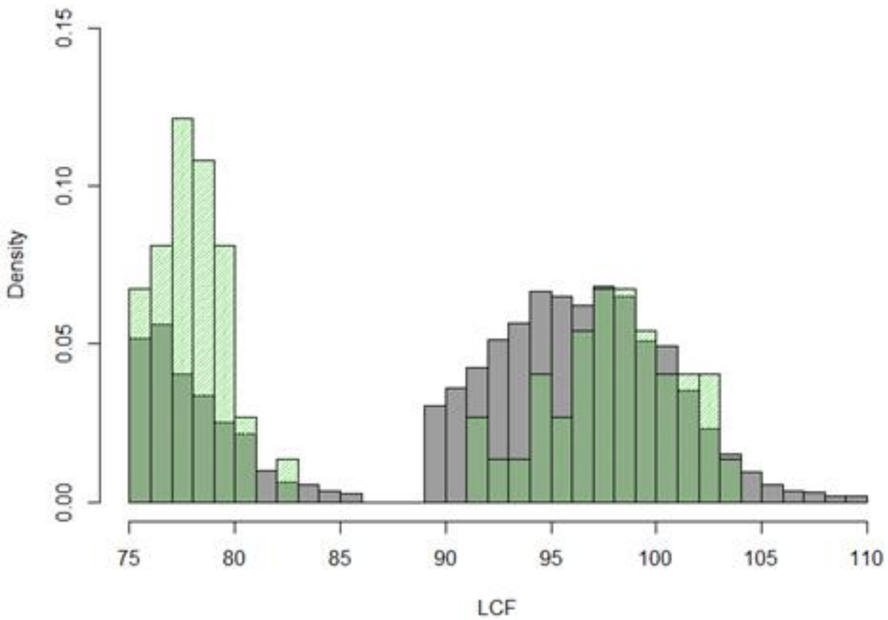

Figure 1: 2022 release and 2023 harvest length distributions for all samples that passed quality control tests (grey) compared to length distribution for samples with a detected match (green). 'Density' on Y axis refers to the matches in green.

Genotyping delays led to the delay of delivery of the 2022 abundance estimate, but this has now been delivered to the 2025 CCSBT data exchange. From 78 million comparisons, 38 matches were detected, and the estimate of abundance is 1.969 million fish in the age-2 cohort (CV = 0.162).

4 Data collection for an estimate of abundance of age-2 cohort in 2023

4.1 Tagging in 2023

In March 2023, the gene-tagging field work team tagged just under 3000 SBT, which is below the target of 5000 fish. The target length range was expanded during the field work to include fish 70–85 cm FL (the usual range is 75–85 cm FL). A large number of small fish were caught by the commercial fishery in that season and a daily examination of the length frequency of the gene-tagging catch (including discarded fish) was used to adjust the length range. The final length range used in the analysis was 70–80 cm FL (inclusive) (Figure 2).

4.2 Harvest sampling in 2024

In 2024, tissue samples were collected from 15,000 fish as they were processed during the harvest from the purse seine fishery in Port Lincoln, South Australia. This is over the 10,000 fish target level of sampling. The additional fish collected at harvest was based on a calculation of the number of samples required to maintain the precision of the estimate. In addition, samples were collected from fish from a wider range of lengths to enable us to detect and adjust to a change in the appropriate length class for age-3 fish. For the final analysis the length range used was 87–101 cm FL inclusive (Figure 2). This length range was chosen to exclude fish from the tails of the length distribution where age classes are likely to overlap (noting that cross-checks between years revealed one of the 105 cm harvest samples is likely to be age 4 as it matched a presumed age-2 tagging sample from 2022).

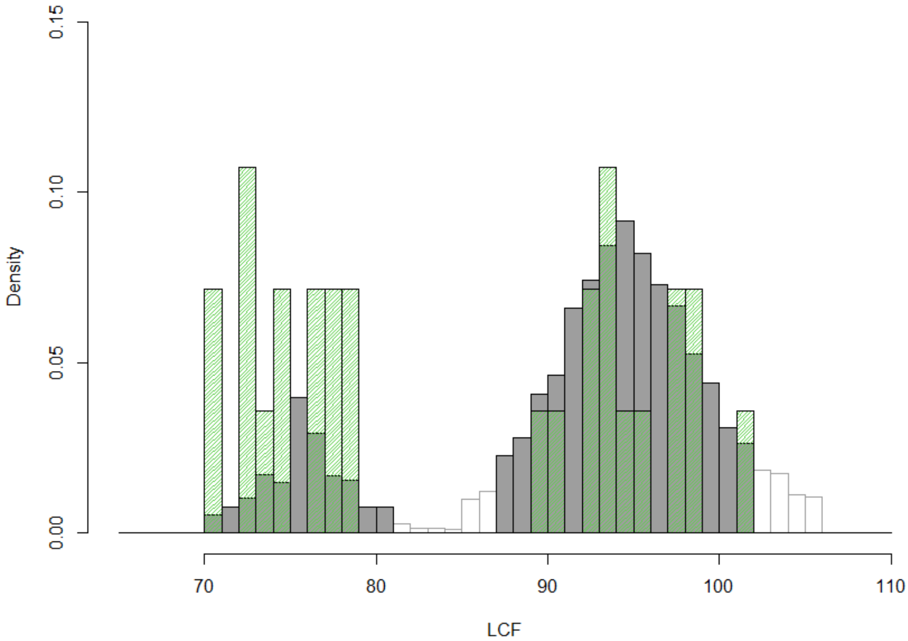

Figure 2: Release and recapture length distributions for all samples used in comparisons (grey) compared to samples detected as a match (green). The white bars indicate samples that were not included in comparisons since they were outside the length ranges chosen to best ensure age-2 fish at release and age-3 fish at harvest. LCF = Lower Caudal Fork length (in cm).

4.3 2023 cohort results

For the final analysis, using the reduced size ranges and excluding data that did not pass quality control checks, there were over 36.7 million genotype comparisons, 14 matches were detected and the estimate of abundance of the age 2 cohort in 2023 is 2.62 million fish (CV 0.267, slightly above the target level of 0.25). 1.1

4.4 DNA extraction 2024

DNA extraction protocols were modified in 2023 -2024 with the purchase of a new robot (liquid handling platform), and the following is an update of lab processing methods:

In the laboratory, biopsy tips were placed into 96-well deep-well plates, and tissue lysis was carried out directly on the biopsy tips. The samples were incubated overnight at 56 °C to ensure thorough breakdown of the tissue and efficient release of nucleic acids.

Following tissue digestion, the lysates were centrifuged in their original 96-well plates to pellet any residual tissue debris. This step ensured that debris would not be transferred, thereby reducing the risk of clogging pipette tips or silica membranes during subsequent purification. The cleared lysates were then transferred to a new 96-well plate using the Integra ASSIST PLUS pipetting robot, which provided consistent sample handling and reduced variability in the pipetting process.

DNA extraction was performed on the Hamilton Microlab STAR automated liquid handling platform equipped with dual on-deck vacuum stations. A custom script was developed specifically to enable ultra-high-throughput, allowing extraction of DNA from up to 960 samples/run. Each 96-well plate contained 92 experimental samples with muscle tissue, 2 control samples from standard reference tissue and 2 blank wells serving as negative controls.

Quality control (QC) testing was performed on 25% of the samples from each plate to evaluate DNA extraction efficiency—including DNA concentration, purity, and integrity—and to assess plate-level consistency. QC wells were selected from three representative columns to capture a spatial distribution across each plate that reflects the overall extraction performance. QC sample preparation was automated using the epMotion® 5075 automated liquid handling system to ensure reproducibility and reduce manual errors.

For each QC assay, 4µL of DNA was used from a total elution volume of 125µL. DNA concentration and purity was measured using the Multiskan SkyHigh spectrophotometer, following a 1:10 dilution of each sample. DNA integrity was evaluated using the Invitrogen Electrophoresis System.

Although QC reliably measures extraction efficiency, it also reflects inherent biological variability at the plate level. This subset-based approach assumes that the average tissue quality within each extraction plate is representative, allowing meaningful inference of overall DNA quality across samples on that plate.

The extracted DNA samples are sequenced using specific SNP markers developed by CSIRO. Quality controls are applied to the genotype data to exclude fish with poor genotypes, or where there is evidence of contamination.

5 Data collection for an estimate of abundance of the age-2 cohort in 2024

Weather conditions during the 2024 field work were challenging. Strong winds throughout the season resulted in extensive upwelling conditions and cooler water temperatures, especially around the areas traditionally targeted by gene tagging. Over 3700 SBT were tagged in the target size range of 70–85 cm FL. This is below the sample size target of 5000 fish. Harvest sampling of 3-yr old fish in 2025 is underway (June-July). The estimate of abundance for the age-2 cohort in 2024 will be available in early 2026.

6 Data collection for an estimate of abundance of the age-2 cohort in 2025 and the reduced gene-tagging program

The CCSBT agreed to a reduced gene-tagging program in 2025 and 2026 because of budget constraints. The reduced program involves fewer days at sea in 2025, and harvest sampling in 2025 and 2026. The numbers of samples processed will be reduced. In 2026 the at-sea tagging component of the program will not be conducted and therefore monitoring of recruitment of the 2026 age-2 cohort will not occur.

In 2025, during the reduced program of 12 days of sea field work time, over 3,600 fish were tagged in the target size range of 70–85 cm FL (see trip report Appendix A). This is below the sample size target of 5000 fish. In 2026 harvest sampling of the 3-yr olds will be conducted.

Tagging will not be conducted in 2026 as agreed under the reduced gene-tagging plan. Therefore, no Research Mortality Allowance is requested for 2026.

7 Logistics

In 2023 and in early 2024, we had problems with some of the genotyping results from 2022 tagging and 2023 harvest sampling and had to have samples re-genotyped. This took time to detect, and resolve, and ultimately delayed the delivery of the 2022 abundance estimate that was due in April 2024 to Dec 2024.

The tips used in tagging were designed and developed to reduce risks of contamination of tissue samples and facilitate fast through-put extraction of DNA (Bradford et al., 2016). In 2016 CSIRO designed an industrial mould to mass produce tips. In 2024, the tips showed signs of degradation of the mould, and CSIRO has commissioned a new mould that is currently undergoing final testing. Following observations of degradation in the biopsy tips, the tissue digestion protocol was revised. The original method was compromised due to burring on the biopsy tips. As a result, lysis was performed directly on the biopsy tips to enable effective tissue digestion and maintain DNA recovery. This modification was implemented to ensure consistency in sample processing and to mitigate the impact of tip degradation on downstream analyses.

Throughout the project, we have used automated liquid handlers to extract DNA from muscle tissue samples. Initially, the Eppendorf EpMotion 5075vt was our primary platform. In 2024, CSIRO commissioned a new Hamilton Microlab STAR automated liquid handler to expand the lab's capacity for DNA extraction and quality control. This upgrade significantly reduced processing time for the ~18,000 samples handled annually, making DNA extractions approximately five times more efficient.

DNA quality control assays are now set up using the EpMotion 5075vt, which has streamlined QC protocols and improved sample tracking precision. The use of automated liquid handlers at this stage has also reduced both time and staff resource requirements.

Both the tip mould and the Hamilton Microlab STAR were purchased by CSIRO through capital expenditure grants.

8 Summary of gene-tagging activity 2016–2025

The gene-tagging program provides the estimates of absolute abundance of the age-2 cohort in the year of tagging. The gene-tagging program's (2016–2025) sample collection and abundance estimates are provided in Table 1. The gene-tagging data will be used in 2025 to run the Management Procedure and review of indicators, and in 2026 for the next assessment of stock status.

Table 1 Genetic tagging programs sample collection 2016–2025. Estimates of the absolute abundance for the age-2 cohort in the year of tagging are available where the full cycle of tagging, harvest sampling, genotyping and analysis has been completed.

YEAR OF TAGGING (Y)	AGE AT TAGGING	N RELEASES	N HARVEST (IN Y+1)	N MATCHES	ABUNDANCE ESTIMATE (MILLIONS)	CV
2016	2	2952	15389	20	2.27	0.224
2017	2	6480	11932	67	1.15	0.122
2018	2	6295	11980	66	1.14	0.123
2019	2	4242	11109	31	1.52	0.180
2020		Interrupted by Covid-19			-	-
2021	2	6401	10742	41	1.68	0.162
2022	2	5084	14714	38	1.97	0.162
2023	2	2759	13297	14	2.62	0.267
2024	2	3730	-	-	-	-
2025	2	3680	-	-	-	-

9 Summary

The 2022 and 2023 estimates of age-2 abundance from the gene-tagging program have been delivered this year and these data will be used in the Cape Town Procedure to recommend the TAC.

The 2024 estimate of abundance is on-track with tagging and harvest tissue samples collected. These will be processed to extract DNA, which will then be genotyped. We expect that the 2024 abundance estimate will be available on time for the 2026 data exchange, for use in the 2026 stock assessment.

Appendix A CCSBT-CSIRO Southern Bluefin Tuna Gene Tagging – March 2025

A.1 Trip report

Report on Gene Tagging Operations for 2025 Field Work.

The field team departed Port Lincoln on 27th March and transited NW to the Cannan Reefs. Searching and fishing for tuna commenced on the morning of the 28th of March and extended until the evening of the 6th of April 2025 (Figure 1). Fishing activities (pole and line) and genetic tagging extended from sunrise to sunset on all active fishing days. Troll lines were deployed each fishing day, except when repositioning the vessel to new fishing grounds. There were two days when the weather was not suitable for the poling and tagging of SBT, and no efforts were made in the search, resulting in 7 days of active fishing from the 12-day charter. The field team returned to Port Lincoln late morning on the 7th of April 2025.

A total of 4,057 SBT were polled; 3,681 of those were in the target size range of 70–85 cm FL (Figure 2) and were tagged (i.e., biopsied) and released. The key tagging locations and tally are provided in Table 1. A further 44 SBT were caught on the troll lines.

Of all SBT caught (pole or troll), 35 were killed as a result of injury sustained during fishing operations, and the rest were released alive. Several SBT returned alive had sustained other injuries not related to our fishing efforts. These SBT were actively feeding and able to take a lure. As such, they were deemed in good condition suitable for return to the water. However, a biopsy was not collected from these individuals.

Biological samples (muscle, vertebrae, heart, otoliths) were collected from each mortality. Length and weight were recorded for all mortalities and troll caught SBT (Figure 3), estimated total weight of mortalities was 344.9 kg.

No other tags (e.g., game fishing tags) were recovered during this field work period.

Table 1. Summary of southern bluefin tuna gene tagged in the 2025 field season.

Area	Approx. Latitude	Approx. Longitude	Number Tagged
Cannan Reefs	-32.644	133.315	1305
Nuyts Reefs (12 mile & 9 mile)	-32.215	131.908	1601
West of Ward	-33.65	133.4	775

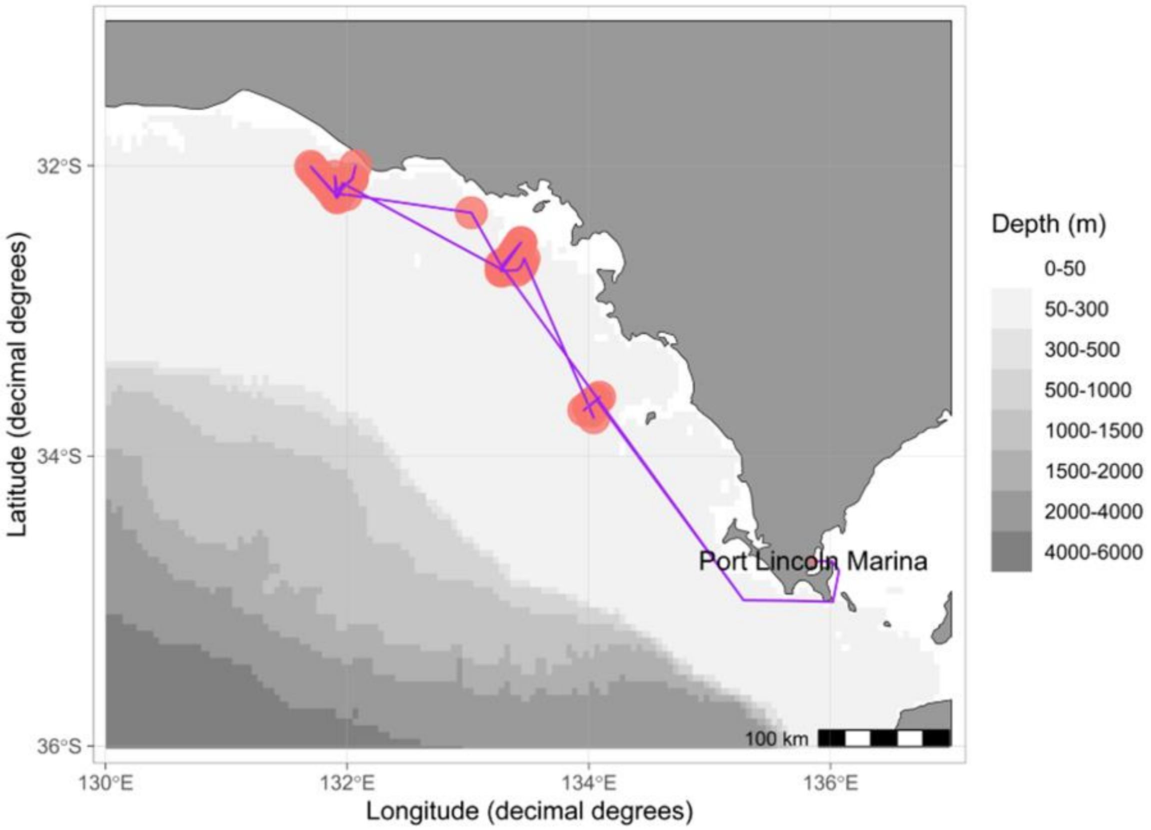

Figure 1 Map of vessel track and tagging locations.

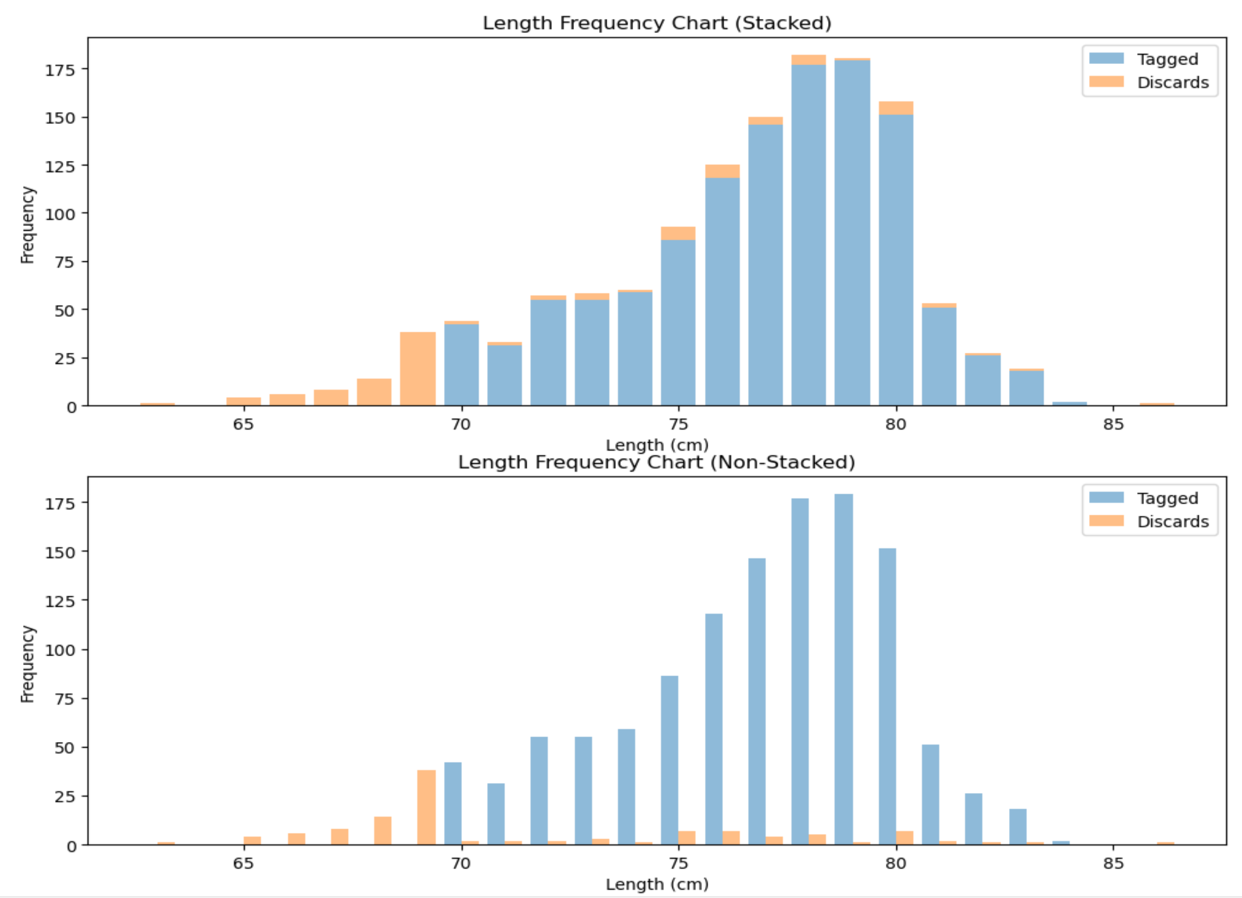

Figure 2. Length frequency histogram of polled southern bluefin tuna during the 2025 gene tagging field operations.

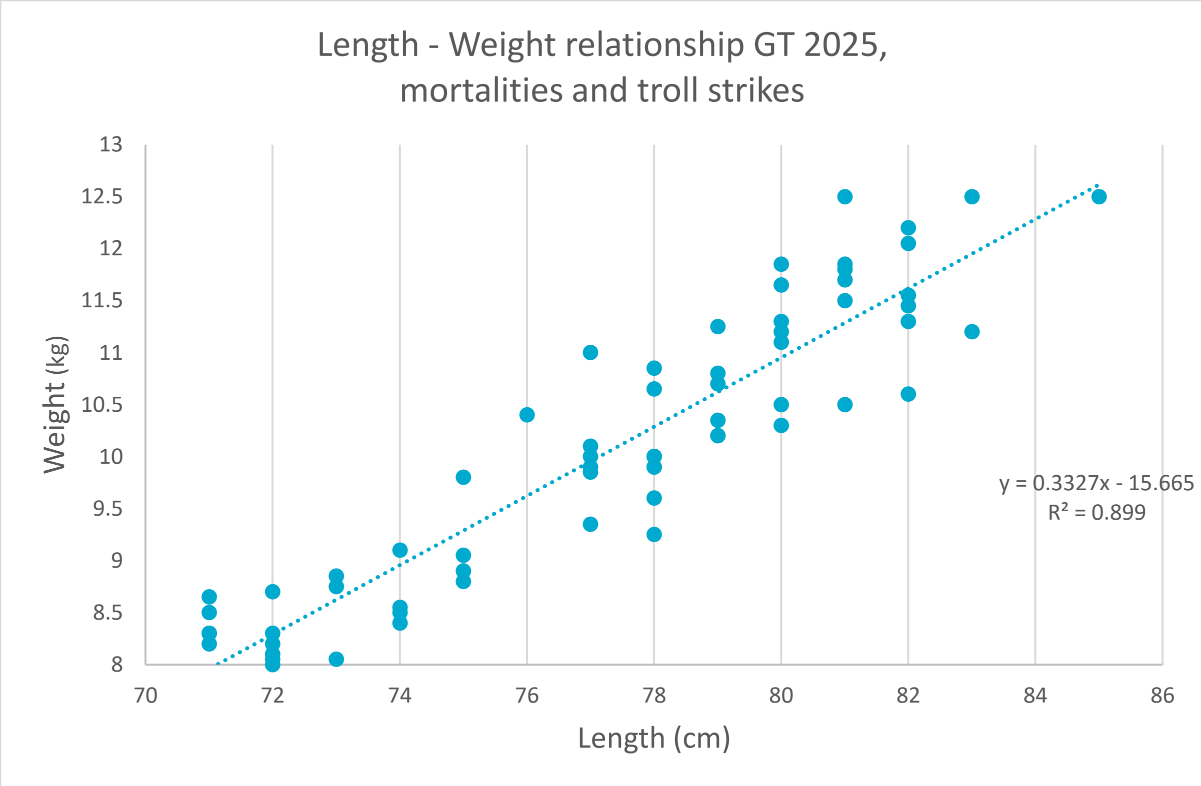

Figure 3. Weight-length relationship of southern bluefin tuna killed as a result of fishing operations in 2025 (n=35) and caught in troll operations (n=44).

Table 2 - Length and weight data for all southern bluefin tuna mortalities collected during the SBT gene tagging field work in March-April 2025.

Date	Mort No	Length	Weight
29/03/2025	1	78	10.85
1/04/2025	2	72	8.05
1/04/2025	3	78	10
1/04/2025	4	72	7.7
1/04/2025	5	78	9.6
1/04/2025	6	78	9.25
2/04/2025	7	82	10.6
2/04/2025	8	78	9.9
2/04/2025	9	72	8.3
2/04/2025	10	69	7.3
2/04/2025	11	80	10.5
2/04/2025	12	80	10.3
3/04/2025	13	78	9.9
2/04/2025	14	77	10.1
3/05/2025	15	74	8.55
3/04/2025	16	75	8.8
4/04/2025	17	79	10.2
4/04/2025	18	74	8.40
4/04/2025	19	79	10.20
5/04/2025	20	73	8.85
5/04/2025	21	82	11.55
5/04/2025	22	68	7.75
5/04/2025	23	75	8.90
5/04/2025	24	81	11.50
6/04/2025	25	81	11.85
6/04/2025	26	71	8.65
6/04/2025	27	82	12.20
6/04/2025	28	83	11.20
6/04/2025	29	80	11.20
6/04/2025	30	78	10.00
6/04/2025	31	80	11.30
6/04/2025	32	72	7.90
6/04/2025	33	80	11.10
6/04/2025	34	76	10.40
6/04/2025	35	82	12.05

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Contact us

1300 363 400
+61 3 9545 2176
csiro.au/contact
csiro.au

For further information

Environment
Ann Preece
+61 3 6232 5336
ann.preece@csiro.au
www.csiro.au/en/research/animals/Fisheries