



# Update on the Gene- tagging program 2024 and RMA request

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Diversity Arrays Technology Pty Ltd.

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# 1 Abstract

The CCSBT 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. The estimate of abundance for the 2022 age-2 cohort is not yet available for the CCSBT Scientific Committee, because there have been delays in genotyping the harvest tissue samples collected in 2023. This delay does not impact running of the Cape Town Procedure (which is scheduled for 2025) or the assessment of stock status (scheduled for 2026).

Here we provide an update on progress on tissue sample collections that will be used in the estimate of abundance of the 2022, 2023 and 2024 age-2 cohorts.

To estimate abundance of the 2022 age-2 cohort, tissue samples were collected from over 5000 aged-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 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 which is being investigated. DNA extraction has been completed, but genotyping was delayed this year. The DNA genotypes from the two sets of samples will be compared to detect matches. The gene-tagging data and results will be provided to the CCSBT later this year.

To estimate the abundance of the 2023 age-2 cohort, 3000 fish were tagged and released in 2023. During the 2024 commercial harvest, over 15,000 samples were collected from a broader length range. These samples will be processed, and genotypes compared to detect matches. The data will be available for the CCSBT data exchange in 2025 and will be used in the Cape Town Procedure.

At sea tagging field work in 2024 occurred over 20 days in March. Over 3700 fish were tagged and released. Research mortality allowance that was used was 271kg. Harvest sampling of age-3 fish will occur in 2025. The abundance estimate for this cohort will be available in 2026 for the next assessment of stock status.

We are seeking 1.5t of Research Mortality Allowance in 2025 for the gene-tagging field work.

## 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 global Total Allowable Catch (TAC), and in the assessment of stock status (e.g. Hillary et al, 2023).

Gene-tagging SBT involves “tagging” fish by 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 tissue samples from tagging and harvesting are processed for DNA extraction and genotyping, and the genotypes from the 2 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 focuses on the data collected in 2022 (tagging) and 2023 (harvest sampling) for calculation of the estimate of abundance of age-2 fish in 2022. The abundance estimate is not yet available because of delays in genotyping the samples. The data and abundance estimate will be provided later this year.

We also provide an update on the gene-tagging programs in 2023 and 2024, Research Mortality Allowance (RMA) usage and the RMA request for 2025. The estimate of abundance, for the age-2 cohort in 2023, will be available in early 2025.

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

### 3.1 Tagging in 2022

Stage 1: tissue sample and release of age-2 fish in the Great Australian Bight during February/March.

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 (75-85cm), and only fish in excellent condition were sampled (tissue collection). 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

Stage 2.1: tissue collection during harvesting, from age-3 fish, during winter (June-August), from fish that were caught by the Australian surface fishery in Jan-Mar.

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. 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 growth. We thank the managers and staff in the processing factories and offshore processing vessels for allowing CSIRO and Seatec access to their facilities, and for their assistance with the project. We thank Seatec for their substantial assistance in liaison with processing factories and the fishing industry for a successful outcome.

### 3.3 DNA extraction results

Stage 2.2: DNA extraction and sequencing using CSIRO SNP markers.

Over 18,000 tissue samples (release 2022 and harvest 2023) were processed using the protocols established for tissue digestion, robotic DNA extraction and quality control. Data on processing, tracking, DNA quality and archiving were collected. As part of the quality control process Nano drop tests and gel electrophoresis were 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 were used as a unique plate identifier, using known DNA for quality/quantity checks. Data were 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 will be sequenced using specific SNP markers developed by CSIRO. Quality controls will be applied to the genotype data to exclude fish with poor genotypes, or where there is evidence of contamination. Genotyping has not yet been completed, and the abundance estimate has not yet been delivered to CCSBT. The gene-tagging data will be provided to the CCSBT (out of usual data exchange timeframe) in 2024 and will be available for use in the management procedure in 2025. Preliminary, but incomplete, results look positive, in that the age-2 cohort is well above the limits used in the Cape Town Management Procedure.



## 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 75-85cm 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. There is work underway to resolve the correct length bin to use, which will inform which of these samples are excluded from the final analysis.

### 4.2 Harvest sampling in 2024

We can compensate for the low number of fish tagged by collecting more samples during the harvest to maintain the CV of the estimate of abundance below the target CV (0.25). Harvest sampling in 2024 has been completed with 15,000 samples collected. A wide length range was used and will be refined, with some samples excluded from final analysis. These samples are now being prepared for DNA extraction and genotyping.

The 2023 age-2 cohort estimate of absolute abundance and the gene-tagging data will be provided to the CCSBT data exchange in 2025 and will be available to use in the Cape Town Procedure.

## 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 resulting in cool water temperatures, especially around the areas traditionally targeted by gene tagging. Over 3,700 SBT were tagged in the target lengths: 70 – 85 cm FL. This is below the target level; however, we can compensate by collecting additional samples during harvest, which is planned for 2025. The field trip report is in Appendix A .

## 6 Summary of gene-tagging activity 2016-2024

The gene-tagging program provides the estimates of absolute abundance of the age-2 cohort in the year of tagging. The gene-tagging programs (2016-2024) 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 in 2026 for the next assessment of stock status.

**Table 1 Genetic tagging programs sample collection 2016-2024. 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. The 2022 estimate of abundance has been delayed but will be delivered in 2024. The 2023 estimate of abundance is on track to be delivered in early 2025.**

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	5000	15000	-	To be delivered in late 2024	-
2023	2	3000	15000	-	To be delivered in early 2025	-
2024	2	3700	-	-	To be delivered in early 2026	-

## 7 Research Mortality Allowance (RMA)

### 7.1 2024 RMA used

The CCSBT agreed to 1.5t of RMA for the 2024 gene-tagging program. The estimated weight of RMA used was 271.1 kg from 22 mortalities. Biological samples were collected from each mortality. Length and weight were recorded for all mortalities (**Error! Reference source not found.**).

### 7.2 2025 RMA request

The request for RMA for the 2025 gene-tagging field trip is 1.5t. This is expected to be an over-estimate of the requirements, that allows for unusual and unforeseen conditions.

## 8 Summary

The 2022 estimate of abundance from the gene-tagging program has been delayed this year because of issues with genotyping. The estimate is expected to be available in the next few months and data will then be provided to the CCSBT. The 2023 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 2023 abundance estimate will be available on time for the 2025 data exchange. The new estimates will be used, with the existing estimates, in the running of the management procedure in 2025 to set recommend the next TAC block.

# Appendix A CCSBT-CSIRO Southern Bluefin Tuna Gene Tagging – March 2024

## A.1 Trip report

This report provides details on the southern bluefin tuna gene tagging field work undertaken by the CSIRO in March 2024.

CSIRO personnel.

Leg 1: Russell Bradford, Emma Westlake: 5– 13 March

Leg 2: Jason Hartog, Jim Dell: 16 –26 March.

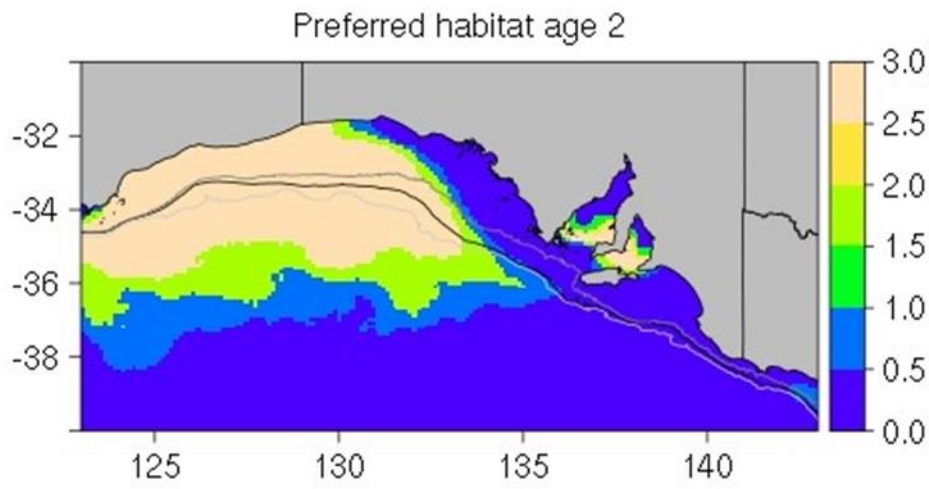
The southern bluefin tuna gene tagging project aims, on an annual basis, to catch, obtain a tissue sample (gene tag), and then release 5,000 southern bluefin tuna (SBT) within the Great Australian Bight. The 2024 gene tagging trip was the 9<sup>th</sup> such trip to tag live SBT.

Commercial fishing operations continued throughout the period of the gene tagging field operations. This restricted the field team's ability to fish without restriction to ensure no overlap between gene tagging and commercial fishing operations. On two occasions the field team were required to depart from an area due to changes in the distribution of the commercial fishing operations.

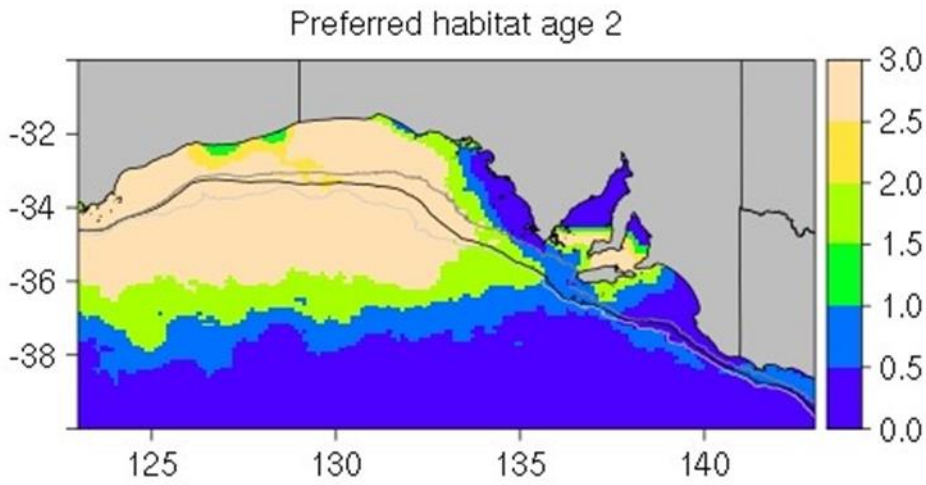
Gene tag fishing operations extended from sunrise to sunset; with processing of mortalities and data entry occurring following the daily end to fishing operations. Weather conditions during the 2024 field work were challenging. Strong winds throughout the season have resulted in extensive upwelling conditions resulting in cool water temperatures, especially around the areas traditionally targeted by gene tagging. Conditions on leg one were the most favourable, with age-2 fish habitat predicted to be closer inshore (Appendix A, Figure 1). By the conclusion of the gene tagging field work, suitable age-2 habitat had retreated considerably to the west (Appendix A, Figure 2).

Fishing operations extended from Kangaroo Island to the head of the Great Australian Bight (Appendix A, Figure 3), covering a total distance exceeding 4,700 km.

A total of 3,733 SBT were gene tagged (5,106 polled). Note that the target fork length was 70 – 85 cm FL in 2024. In contrast to the previous few years, the majority of SBT outside of the target range were above 85 cm FL (Appendix A, Figure 4). The key tagging locations and tally are provided in Appendix A, Table 1. A further 33 SBT were caught on the troll lines. Of all SBT caught (pole or troll), 22 were killed as a result of injury sustained during fishing operations. Biological samples were collected from each mortality. Length and weight were recorded for all mortalities and trolled SBT (Appendix A, Figure 5); estimated total weight of mortalities was 271.1 kg.

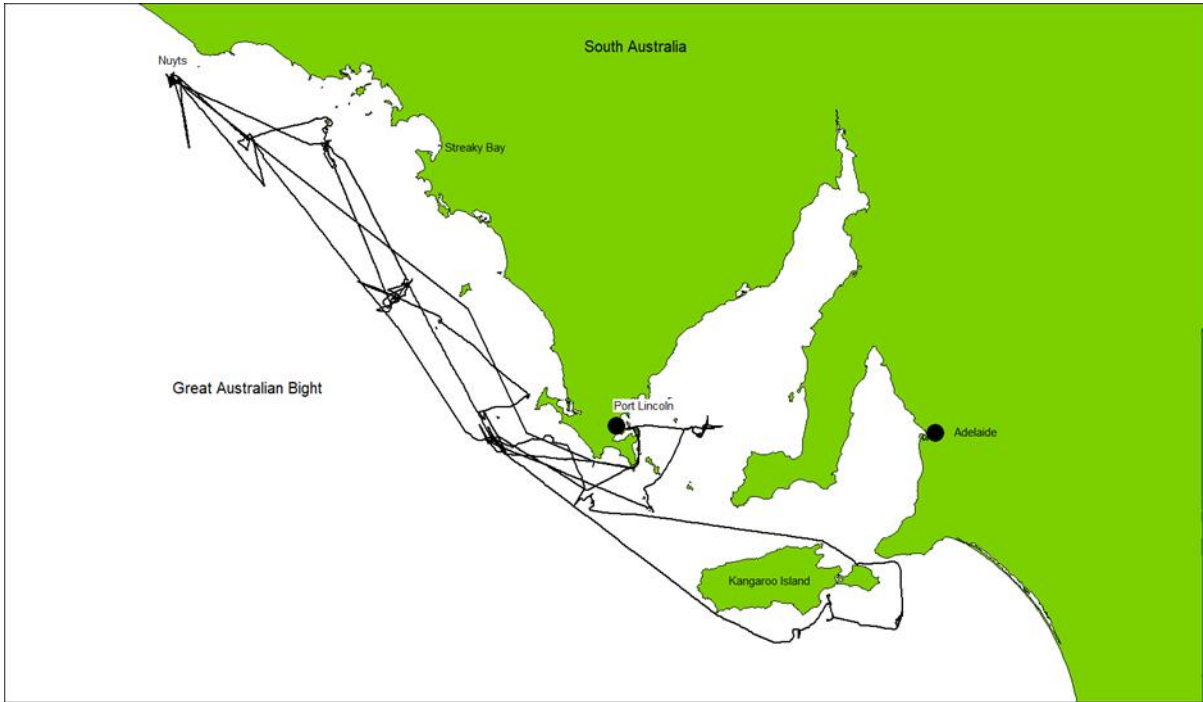


Appendix A, Figure 1. Habitat prediction model for age-2 southern bluefin tuna on 06 March 2024.

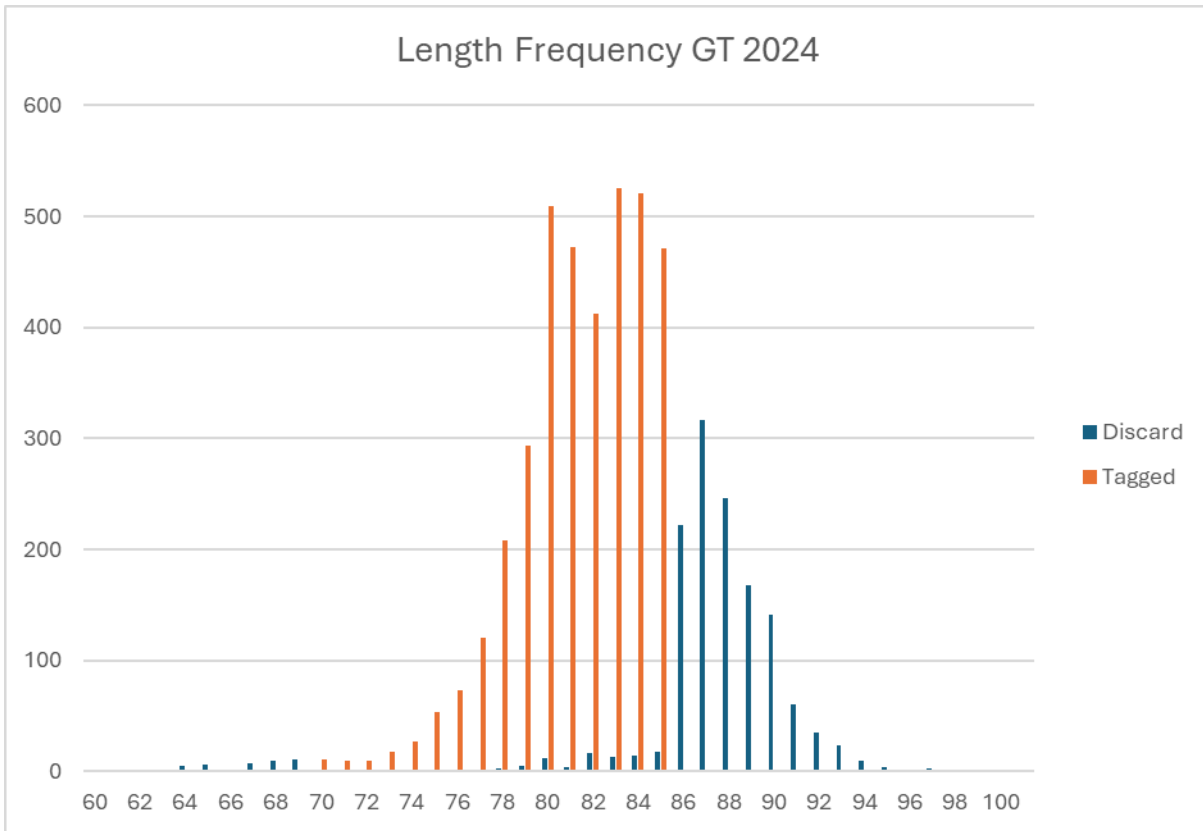


Appendix A, Figure 2. Habitat prediction model for age-2 southern bluefin tuna on 26 March 2024.

Map of the track of the vessel from Port Lincoln in the Great Australian Bight



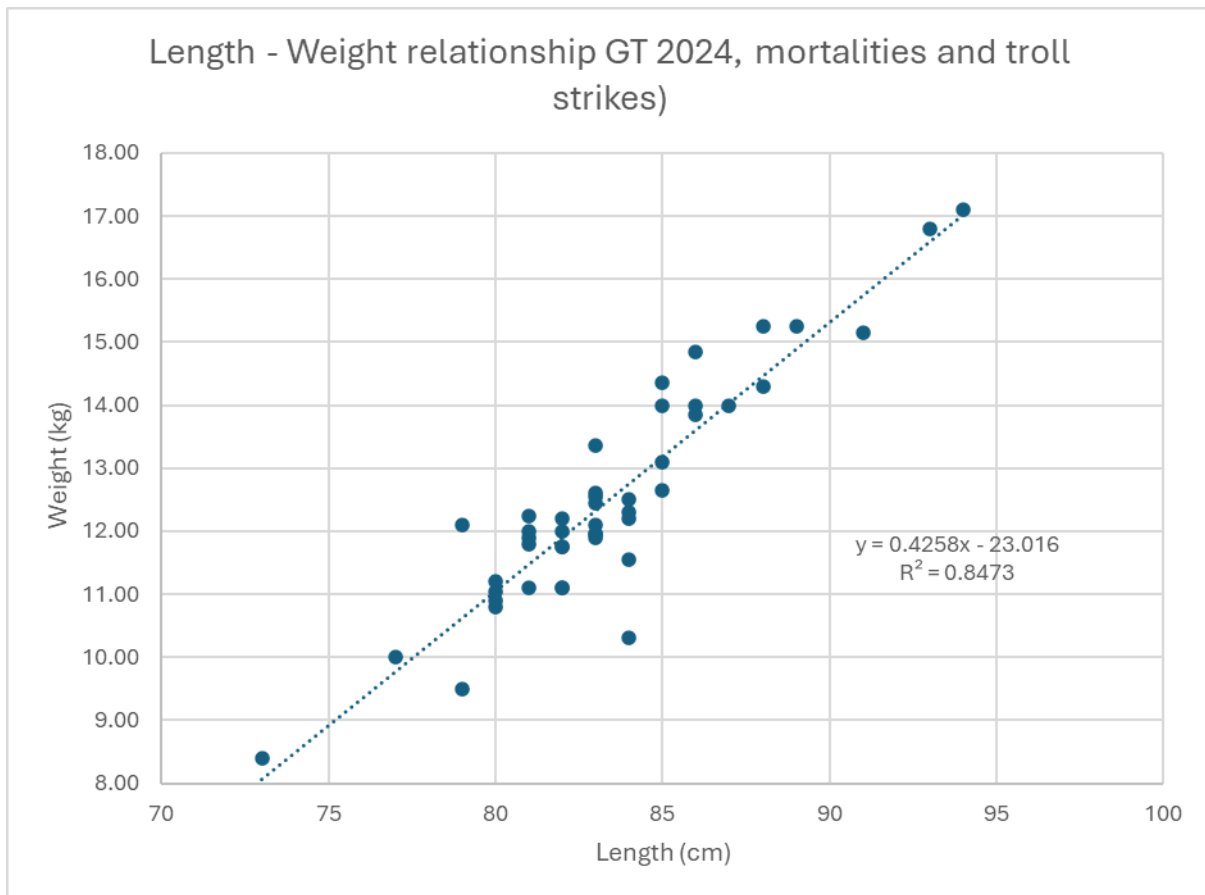
Appendix A, Figure 3. The track of the Yasmin during the 2024 Gene Tagging field work.



Appendix A, Figure 4. Length frequency histogram of polled southern bluefin tuna during the 2024 gene tagging field operations.

Appendix A, Table 1. Summary of southern bluefin tuna gene tagged in the 2023 field season.

Area	Approx. Latitude	Approx. Longitude	Number Tagged
Rosalind (Spencer Gulf)	-34.705	136.609	255
Rocky Island	-34.779	134.731	888
Cannan Reefs	-32.644	133.315	120
Nuyts Reefs	-32.215	131.908	2,470




Appendix A, Figure 5. Weight-length relationship of southern bluefin tuna killed (mortalities) and caught on troll lines for the 2024 gene tagging field operations

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