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Update on the Gene-tagging program 2023 and RMA request

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Acknowledgements

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. Five estimates are available for the age-2 cohorts in 2016-2019 and 2021. The estimate of abundance of fish aged-2 in 2021 is 1.68 million, and coefficient of variation (CV) is 0.16. This is an increase in abundance compared to the previous estimate. Tissue samples were collected from over 7000 aged-2 fish in 2021, and the fish were released alive. Over 11,000 tissue samples were collected from age-3 fish in 2022 during harvest in South Australia. The DNA genotypes from the two sets of samples were compared to detect matches. Over 68 million DNA comparisons were made, and 41 matches detected. The gene-tagging data and results were provided to the CCSBT data exchange in early 2023. The 2022 and 2023 field work trips have been completed with over 5000 fish tagged in 2022, and under 3000 fish tagged in 2023. The 2023 harvest sampling logistics were more complex this year with tissue samples collected during off-shore processing. Over 15,000 harvest samples have been collected from a wider length range, to allow for a potential shift in length at age which will be investigated before calculating the next (i.e., 2022) estimate of abundance.

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 2023 stock assessment (Hillary et al, 2023). These data are also used in the Cape Town Procedure (Anon., 2020) to provide a management recommendation on global Total Allowable Catch (TAC).

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 fish 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. 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 (Preece et al., 2015). Twenty days at sea is 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 2021 (tagging) and 2022 (harvest sampling) for calculation of the estimate of abundance of age-2 fish in 2021. These data were provided to the CCSBT data exchange in 2023.

We also provide an update on the gene-tagging programs in 2022 and 2023, Research Mortality Allowance (RMA) usage and the RMA request for 2024. The next estimate of abundance, for the age-2 cohort in 2022, will be available in early 2024.

3   Estimate of abundance of age-2 cohort in 2021

3.1   Tagging in 2021

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

In 2021, the gene-tagging field team collected over 7,000 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). The 2022 sample collection exceeded the target of 5000 fish. This was the 6th gene-tagging field trip (noting 2020 was cancelled mid-trip due to COVID-19 and poor fishing).

3.2   Harvest sampling in 2022

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 2022, tissue samples were collected from 11,000 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 2022 this involved:

•   Collection of tail stalks, which are removed as part of normal processing, and labelled with the record length of the fish. Date of harvest, length, collector and other details are also recorded. The tail stalks were frozen for later tissue biopsy.

•   Collection of tissue samples from the tail stalks. Frozen tail stalks are thawed slightly, and a tissue biopsy taken. 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).

All participating factories were visited during the harvesting season. The sampling is designed to select fish at random from across the different farms and processing factories, and throughout the full harvest period. Only fish within a restricted length (size classes for age-3 fish) are selected. 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. More fish were processed off-shore in 2022, which made the logistics of on-shore sample collection more difficult. We thank Seatec for their substantial assistance in liaison with processing factories 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 and harvest) 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 were sequenced using specific SNP markers developed by CSIRO. Genotyping was completed in early 2023. Quality controls were applied to the genotype data to exclude fish with poor genotypes, or where there was evidence of contamination. Of the samples genotyped, 96% were used in the analysis.

3.4   The 2021 estimate of abundance

Stage 3: calculation of the estimate of abundance (of the age-2 cohort in 2021).

The estimate of absolute abundance for the age-2 cohort in 2021 is 1.68 million fish. This is an increase from the previous estimate. The number of tagging and harvest samples positively exceeded the design study target sample sizes (5,000 releases and 10,000 harvest samples). This has resulted in a more precise estimate (CV 0.16) than the design study target CV of 0.25. Over 68 million comparisons were made resulting in 41 matches being detected. The estimate of abundance was provided to the CCSBT data exchange in April 2023.

4   Results for 2016-2021

The results of the 2016-2021 gene-tagging program provide the estimates of absolute abundance of the age-2 cohort in the year of tagging (**Error! Not a valid bookmark self-reference.**). The gene-tagging data used in the 2023 stock assessment are the year of release, age of release, year of recapture, number of releases, number of harvest samples and number of matches.

Table 1 Results of the 2016-2021 genetic tagging programs, which provide an estimate of the absolute abundance for the age-2 cohort in the year of tagging.

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

5 Progress for next estimates of abundance

5.1 Progress on the 2022 estimate of abundance

Stage 1 tagging:

In March 2022, the gene-tagging field team collected tissue samples from over 5,000 SBT, in the 7th field season.

The target fork length in 2022 was between 75 and 85 cm (FL). The fork length of SBT tagged peaked at 76 cm (Figure 2). The number of SBT caught and returned without sampling (i.e., those outside the target FL) was 3,242 and skewed to fish smaller than the target range (Figure 1).

This shift in the length frequency could be due to annual variation or slower growth in juvenile fish. From the 1960s-1990s there was an increase in growth which was detected from the conventional tagging studies, and decadal change in growth is incorporated into the SBT operating models. A hypothesis for the increase in growth was that it was a response to the decreasing population size in this period. If slower growth is occurring, it could be a response to the recent increase in population size, or other drivers. We are examining direct age data (from otoliths and vertebrae) to fine tune the length range for gene-tagging and hope to report on this next year.
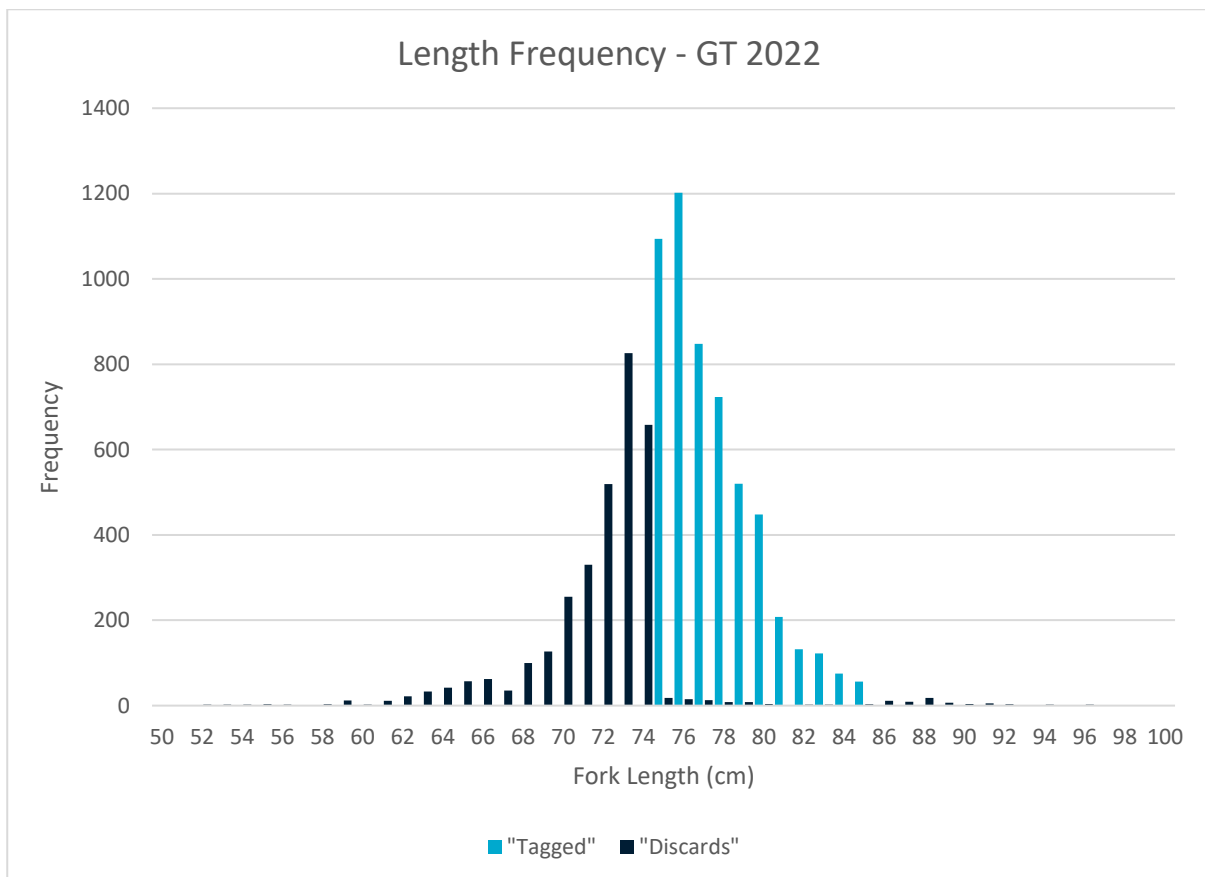

Figure 1 Length frequency histogram of pole-caught SBT during the 2022 gene tagging field season. Blue = tagged SBT; orange = caught and released without tagging ("discards").

Stage 2: harvest sampling.

In June-July 2023, over 15,000 tissue samples were collected during the purse seine harvest in Port Lincoln, thanks to substantial cooperation from the SBT Industry Association member, processors and their staff, and Seatec. This 2023 gene-tagging tissue sample collection occurred both on-shore and off-shore. A wider length range was adopted for harvest sample collection, to ensure that collected samples are from age-3 fish if slower growth is occurring. We will refine the length range to be used during the analysis stage, which is likely to result in exclusion of some samples. The additional samples collected (over the target of 10,000) gives us a buffer if large numbers are excluded from the analysis.

These samples will now be processed at the CSIRO labs in Hobart and genotyped. The next (i.e., 2022) estimate of abundance will be available in early 2024.

5.2 Progress on the 2023 estimate of abundance

Stage 1: tagging

In March 2023 the gene-tagging field work team tagged just under 3000 SBT, which is below the target of 5000 fish (trip report, Appendix A . The target length range was expanded during the field work to include fish 70 – 85 cm FL (the usual range is 75-85cm FL). This was a result of the high number of small fish being caught in the current season and was informed by daily examination of the length frequency (Appendix A Figure 2) of the gene-tagging catch (including discarded fish). The work to resolve the correct length bin to use, will inform which of these samples are excluded from the final analysis.

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).

6　Research Mortality Allowance (RMA)

6.1　2023 RMA used

The CCSBT agreed to 1.5t of RMA for the 2023 gene-tagging program. The estimated weight of RMA used was 359.7kg from 44 mortalities. Biological samples were collected from each mortality. Length and weight were recorded for all mortalities (Appendix A Figure 3).

6.2　2024 RMA request

The request for RMA for the 2024 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.

7　Summary

The CCSBT gene-tagging program has successfully completed five full cycles (2016-2019 and 2021), except in 2020 when the field work was interrupted by COVID-19 travel restrictions and the remainder of that cycle did not proceed. The 2016-2021 estimates of abundance are used in the 2023 full stock assessment for assessing current stock status.

In 2021 over 7000 fish were tagged and samples were collected from over 11,000 fish during the harvest in 2022. DNA was extracted from all suitable tissue samples. The estimate of abundance is 1.68 million fish in the 2021 age-2 cohort which is an increased from the previous estimate and is more precise (CV 0.16).

Appendix A CCSBT-CSIRO Southern Bluefin Tuna Gene Tagging – March/April 2023

A.1   Trip report

This report provides the details the southern bluefin tuna gene tagging field work undertaken by the CSIRO in March/April 2023.

CSIRO personnel:

- Leg 1: Russell Bradford, Emma Westlake, 15 – 26 March

- Leg 2: Jason Hartog, Naomi Clear, 26 March – 6 April.

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 2023 gene tagging trip was the 8th such trip to tag live SBT.

Commercial fishing operations extended well into March 2023 resulting in a mid-March departure to begin gene tagging fieldwork. Unfortunately, all fishing operations were not completed by the time of the start of gene tagging. The team were, therefore, restricted to working well west of commercial fishing operations for the majority of the first leg of fieldwork. The field of operations for leg two were unrestricted.

Fishing activities extended from sunrise to sunset on all active fishing days. Of the 20 fishing days in the field, seven days resulted in no fish being caught and tagged. Figure 1 shows the track the vessel took while searching for southern bluefin tuna (SBT). A total of 2,970 SBT were gene tagged (4640 polled), with 1,690 on leg one and 1,280 on leg two. Note that the target fork length was expanded from 75 – 85 cm FL in previous years to 70 – 85 cm FL in 2023. This was as a result of the high number of small fish being caught in the current season and was informed by daily examination of the length frequency (see figure 2) of the catch (including discarded fish). The key tagging locations and tally are provided in Table 1. A further 137 SBT were caught on the troll lines. Of all SBT caught (pole or troll), 44 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 (Figure 3), estimated total weight of mortalities was 359.7 kg.
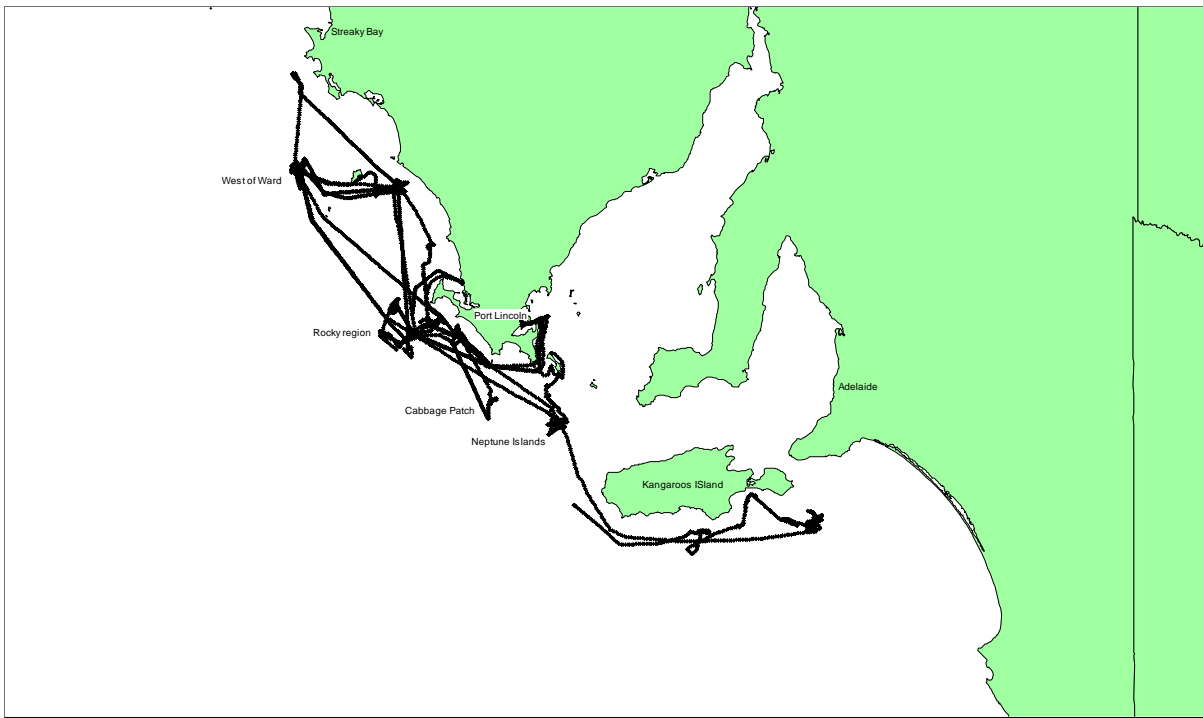
Appendix A Figure 1. The track of the Yasmin during the 2023 Gene Tagging field work.

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

Area	Latitude	Longitude	Number Tagged
Neptune Islands	-35.284	135.984	644
Rocky Island region	-34.831	134.771	152
Sanders Bank	-35.955	138.385	149
Stewarts Reef	-34.836	135.373	136
Top Gallant	-33.771	134.597	1006
West of Ward	-33.816	133.838	883
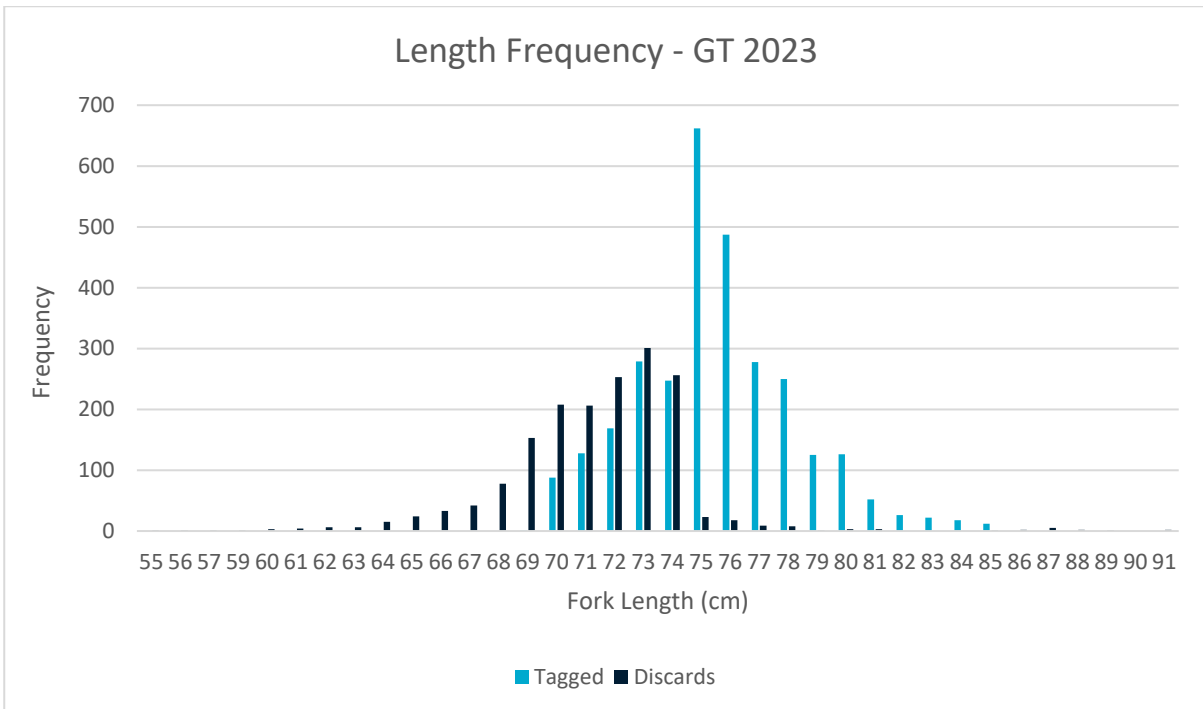
Appendix A Figure 2. Length frequency histogram of polled southern bluefin tuna during the 2023 gene tagging field operations.
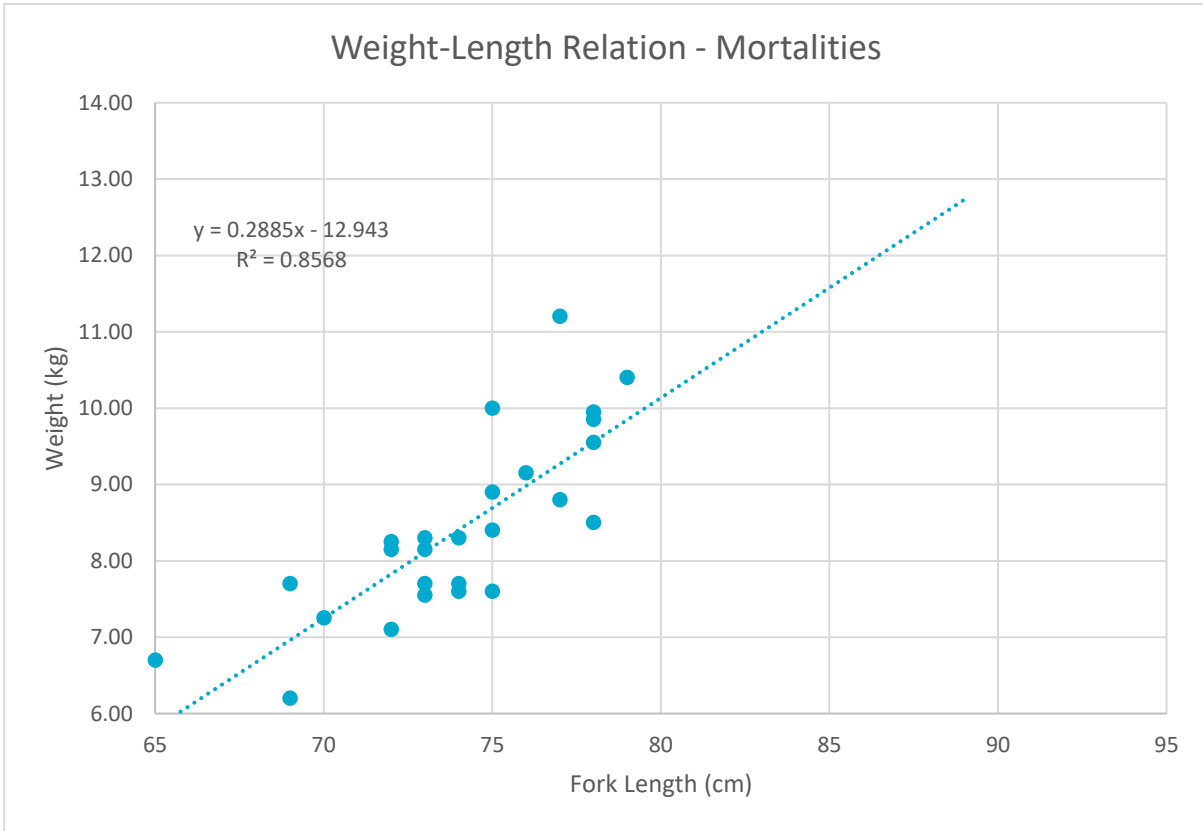
Appendix A Figure 3. Weight-length relationship of southern bluefin tuna killed as a result of fishing operations in 2023.

References

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