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Report of the SBT gene-tagging program 2021

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Report to the Commission for the Conservation of Southern Bluefin Tuna

Contents

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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 2019 abundance of age 2 fish is calculated from the number of fish tagged and released in 2019, the number of 3-year-old fish sampled during harvest in 2020, and the numbers of matches (analogous to a tag recapture) detected from genotype analysis of DNA from the tissue samples. The analysis found 31 matches from over 47 million comparisons across the tagging and harvest data sets. The estimate of abundance of the age 2 cohort in 2019 is 1.52 million fish (CV 0.18). This abundance estimate is higher than the estimates of abundance of age 2 fish in 2017 and 2018, and well above the estimates from the years corresponding to very low recruitment in the stock assessment models (1999-2002). There will not be an estimate of abundance provided next year, because the 2020 tagging field work was cancelled due to of COVID-19 restrictions, poor weather and difficulty finding fish. The 2021 tagging work has, in contrast, been very successful with over 7100 fish tagged and released. The completed data sets and 2019 abundance estimate have been provided to the CCSBT scientific data exchange. The 2016-2019 abundance estimates will be used in the Cape Town Procedure in 2022 for recommending the total global allowable catch for the period 2024-2026.

2 Introduction

The CCSBT gene-tagging program is designed to provide an estimate of the absolute abundance of age 2 SBT, for use in the Cape Town Procedure and stock assessment models. The program has been in operation since 2016.

This report provides the latest estimate of the abundance of age 2 fish in 2019, calculated from the tag and release field work in 2019 and the harvest sampling in 2020.

We also provide an update on the gene-tagging programs in 2020 and 2021, Research Mortality Allowance (RMA) usage and the RMA request for 2022.

3 Method

Gene-tagging SBT involves "tagging" fish by taking a very small tissue sample (Bradford et al 2016) 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 DNA from the two sets of samples are genotyped and then compared in order to find the samples 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 sources of bias, costs and precision of estimates and integration of data in stock assessment and management procedure models (Preece et al., 2015).

The 2019 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 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.

The project involved the following steps:

1. Tag and release: vessel charter and pole and line fishing for at-sea collection of tissue samples from age 2 fish in the Great Australian Bight during the summer of year 1 (2019).
2. Harvest sampling: collection of tissue samples from age 3 fish in winter of year 2 (2020), during harvest of fish in farms, which were caught by the Australian surface fishery in the summer 2020.
3. DNA extraction and genotyping of tissue samples, using CSIRO-developed SNP markers.
4. Data analysis and calculation of an abundance estimate, and provision of results to the CCSBT data exchange and Extended Scientific Committee for use in stock assessment models and the management procedure.

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, given they are selected using a specified length-class. Direct ageing of otoliths and vertebrae was used in 2019 to revise length classes chosen to target 2-year-olds and 3-year-olds (Preece et al., 2019).

4 2019 gene-tagging results and discussion

4.1 Tag and release - tissue collection 2019

The 2019 tagging program tagged and released 4631 fish during the vessel charter (Figure 1). The commercial season finished late, and it was difficult to find fish in the size classes required. Results from the tagging component of the project were reported to the Extended Scientific Committee in 2019 (Preece et al., 2019).

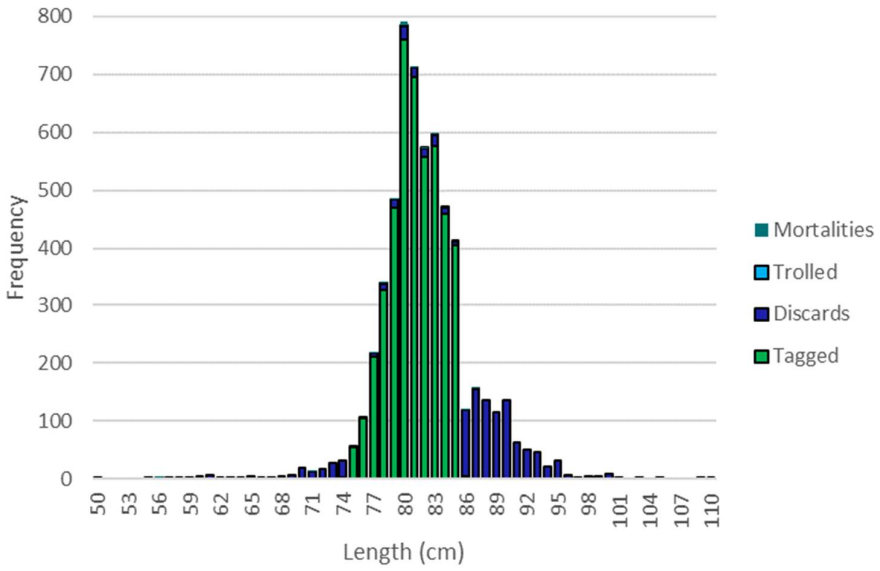

Figure 1 Length frequency of SBT tagged (in target range, green) and discarded (out of range, blue) in 2019.

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 or less; quicker than conventional tagging methods, and considerably faster and less invasive than archival tagging methods. The DNA samples have been safely archived at CSIRO, Hobart. Tagging data are managed in a database and are provided to the CCSBT data exchange.

4.2 Tissue collection during harvest in 2020

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 marked for later identification. Date of harvest, length, collector and other details are recorded. The tail stalks are 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 and loaded into individually labelled vials. Additional data are recorded (e.g. date, collector, sample number).

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 12,000 tissue samples were collected from fish in the target length range, well in excess of the design study target of 10,000 samples, and all were processed to extract DNA.

4.3 DNA extraction and sequencing, using CSIRO SNP markers

Nearly 17,000 tissue samples (release and harvest) have been processed using established protocols 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 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 ultra-low freezer. Data are recorded during all stages of the processing, to note unusual samples or results, errors or changes from original storage 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 two control samples. A small percentage of samples had poor quality or quantity of DNA and, therefore, were not successfully sequenced, although the overall success rate was very high (>93%).

4.4 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 of each individual). This involved first filtering the data to exclude fish with incomplete or poor genotype information (too few SNP markers with good sequencing results). Any fish outside the target release (75-85 cm) and harvest (98-109 cm) length ranges were also excluded. A fish was determined to have been recaptured if there was a fish with a matching set of markers in both the release and harvest sample sets. The analysis of releases against harvest samples involves approximately 47 million comparisons. The

analysis has identified 31 matches (recaptures). The estimate of abundance of age 2 fish in 2019 is 1.52 million with coefficient of variation (CV) of 0.18 (Table 1), which is within the range initially considered during the design project. This abundance estimate is larger than the most recent estimates of 1.15 and 1.14 million fish in 2017 and 2018, respectively. It is larger than estimates for the age 2 cohorts (2002-2004) from the years of very low recruitment in the stock assessment models (2000-2002) and is close to the predicted estimate from the stock-recruitment function in the 2020 SBT operating models (Figure 2). Data were provided to the CCSBT scientific data exchange.

Table 1 The results of the gene-tagging programs 2016-2018 which provide the absolute abundance estimate for the age-2 cohort in the year of tagging.

YEAR	COHORT AGE	N RELEASES	N HARVEST	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

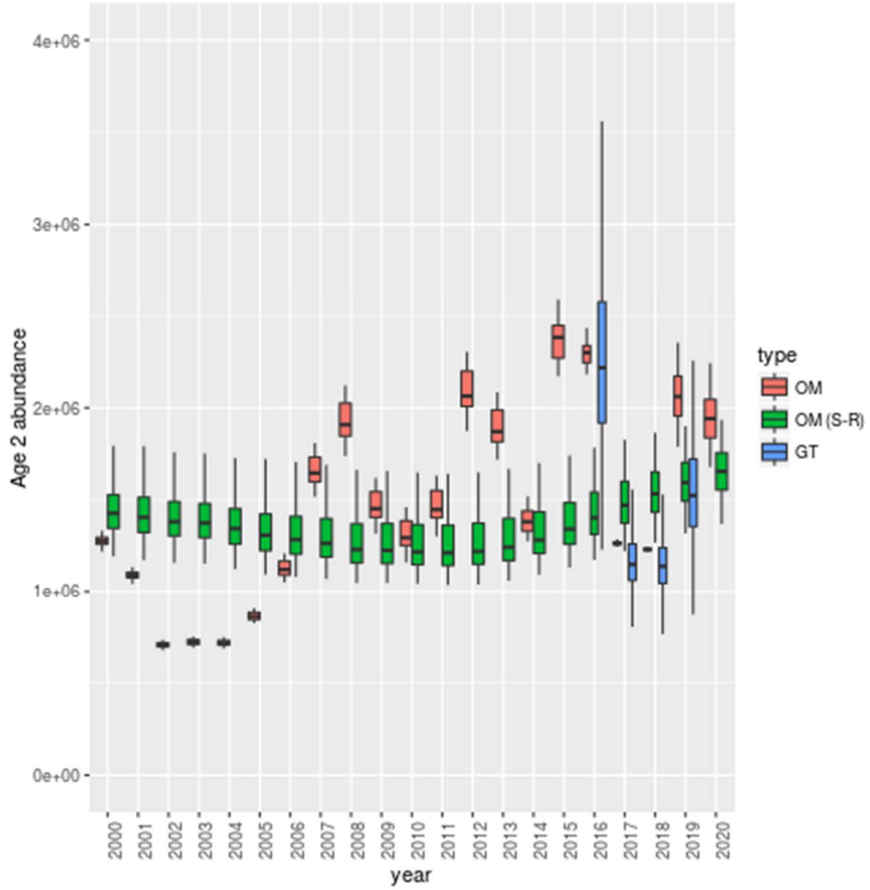

Figure 2 Comparison of 2016-1019 gene-tagging age-2 abundance estimates (blue) and recent age-2 estimates from the 2020 reconditioning of the OM (red) and those predicted from the stock-recruitment function (OM-(S-R)) (green). The 2019 gene-tagging abundance estimate has not been included in the OM reconditioning.

The gene-tagging data used in the stock assessment models are the year of tag and release, age of fish at time of release, year of harvest sampling, the number of releases, number of harvest samples, and the number of matches (Hillary et al 2020a). The gene-tagging data used in the Cape Town Procedure are the number of matches, the abundance estimate, and the age and year to which the estimate applies (Hillary et al, 2020b).

5 The 2020 gene-tagging program

In 2020, the fifth cycle of gene-tagging commenced with at-sea tagging in March 2020. The field team had difficulties finding fish, and weather conditions were not ideal. The CSIRO field team was urgently recalled back to Hobart after only 9 days of the 20-day field trip because of COVID-19 risks and border closure uncertainties at that time. Too few fish were sampled in the limited time at sea to provide a sufficient release sample size for abundance estimation, and therefore there was no harvest sampling in 2021; thus, the gene-tagging program will not deliver an estimate of abundance in 2022.

6 The 2021 gene-tagging program

In 2021 the tagging field work recommenced, and 7155 fish were tagged and released, substantially more than the 5000 fish target. A trip report is provided in Appendix A: 2021 Field Trip Report Harvest sampling will recommence in 2022, and the data and abundance estimate will be available in early 2023.

7 Research Mortality Allowance

None of the 2020 RMA was used during the shortened field work.

In 2021 310kg of RMA was used. There were 34 mortalities (see trip report, Appendix A).

The request for RMA for the 2022 field trip is 2t. This is expected to be an over-estimate of the requirements, that allows for unusual and unforeseen conditions.

8 Summary

The fourth full-cycle of the CCSBT gene-tagging program has been successfully completed, with over 4800 fish tagged in 2019 and samples collected from over 12,000 fish during the harvest in 2020. 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 analysis found 31 matches from over 47 million comparisons across the tagging and harvest data sets. The abundance estimate for the age 2 cohort in 2019 is 1.52 million fish (CV 0.18). The complete data sets and abundance estimate have been provided to the CCSBT scientific data exchange. The 2016-2019 abundance estimates will be used in 2022 in the management procedure for recommending the total global allowable catch.

The 2020 tagging field work was cancelled after only a few fish were tagged, and the field team were recalled to Hobart because of COVID-19 restrictions. As a result, harvest sampling did not proceed in 2021 and there will not be a gene-tagging program estimate of the 2020 age-2 cohort in 2022.

The 2021 tagging field work has been very successful with over 7100 fish tagged and released, and harvest sampling will recommence in 2022. The 2021 abundance estimate will be available in early 2023 for use in the next stock assessment.

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Appendix A: 2021 Field Trip Report

CCSBT-CSIRO Southern Bluefin Tuna Gene Tagging – March/April 2021

CSIRO Personnel: Russell Bradford, Jason Hartog, Kylie Maguire

Trip dates: 23 March 2021 to 13 April 2021.

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 2021 gene tagging trip was the 6th such trip to tag and release live SBT.

Commercial fishing operations extended well into March to due to poor weather conditions and other factors, delaying the start to the Gene Tagging project field work. Despite several commercial fishing operations still in progress, the gene tagging team (JH & RB) departed Port Lincoln on the 22nd March 2020. The team headed first to Stewart's Reef (west of commercial operations) where reports of large schools had been observed by the professional aerial spotter. Over the course of the first leg of field work, weather conditions were ideal for spotting SBT. The first leg extended to 11 days with 6,624 SBT gene tagged during that period.

The second leg of field work commenced on 06 April 2021 with Kylie Maguire replacing Jason Hartog. Weather conditions progressively deteriorated during the second leg of field work making SBT spotting difficult. During the nine days of the second leg a further 531 SBT were gene tagged, for a total of 7,155 SBT gene tagged in 2021, making the 2021 season the third most successful gene tagging trip to date.

The Yasmin traversed a minimum of 4,100 km during the 2021 field work (Figure 3) covering the key areas where SBT are typically found (see Appendix A for past vessel tracks). Despite wide coverage, fishing operations were concentrated in areas to the west and south of the Eyre Peninsula, specifically: Stuart Reef (2,578 tagged), Cabbage Patch (1,716 tagged), and Price Island (2,457). Sampling of these locations occurred throughout the 20 days to allow time for fish to move through and avoid sampling from the one school. Smaller numbers were tagged at the eastern areas of Pelorus, Dragies and near Tunk Head.

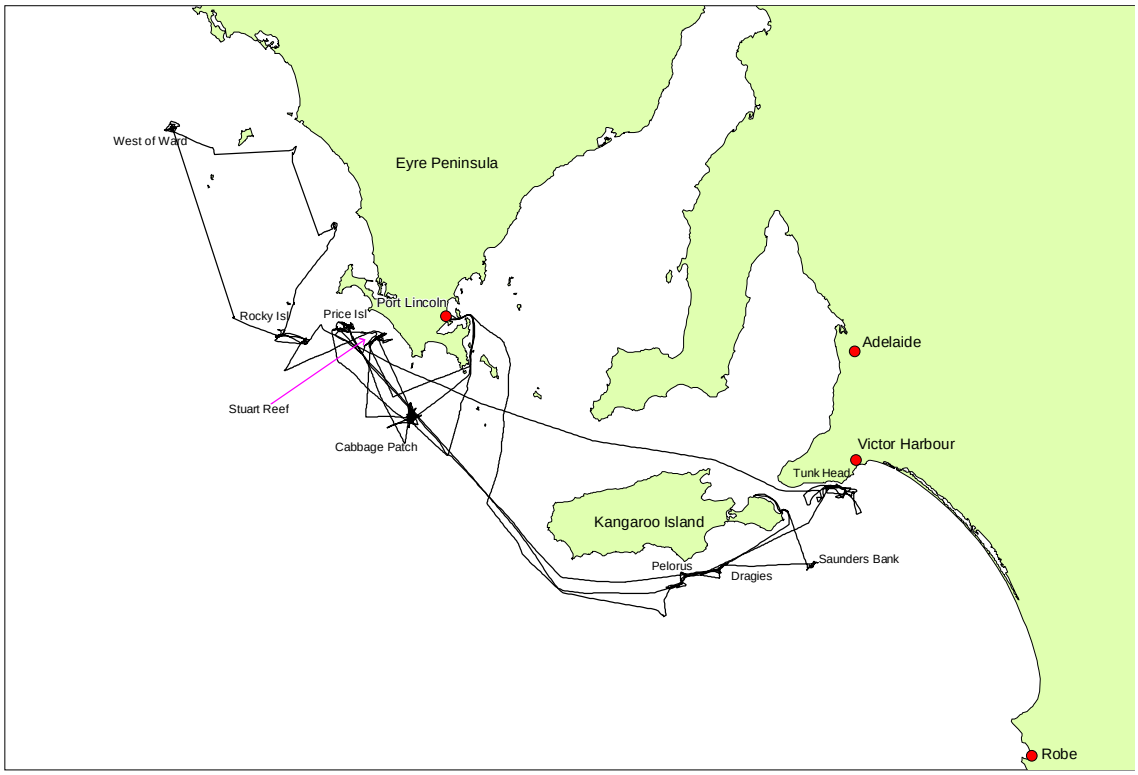

Figure 3 The track of the Yasmin during the 2021 Gene Tagging field work. Distance covered: approx. 4,100 km

The target fork length in 2021 was between 75 and 85 cm (FL). The fork length of SBT tagged peaked at 80 cm (Figure 4). The number of SBT poled and returned without sampling (i.e. those outside the target FL) was 1,229 and skewed to fish smaller than the target range (Figure 5).

A total of 156 SBT were caught on the troll lines while searching for schools of SBT. Of these, three were killed because of injury sustained from the troll line; the remaining 153 were returned after being measured and weighed. The length-weight relationship for the troll caught SBT is provided in Figure 6.

Thirty-one SBT sustained damage from the poling/trolling operations that was deemed fatal. These fish were immediately humanely killed and biological samples obtained from them. Additionally, these fish were used to train in archival tag insertion and suturing techniques. Total mortalities amounted to approx. 310 kg of SBT.

At all times while sampling, a temperature logger was in the eski that was used on deck to store tissue samples prior to being placed into the freezer. Typically, samples would not be in the deck eski for longer than approx. 30 minutes. The deck eski is loaded with ice blocks at the start of the day when fishing operations begin. It is cleared of ice blocks and cleaned at the end of the day. During sampling periods, the temperature remained relatively stable between 3 and 4 °C.

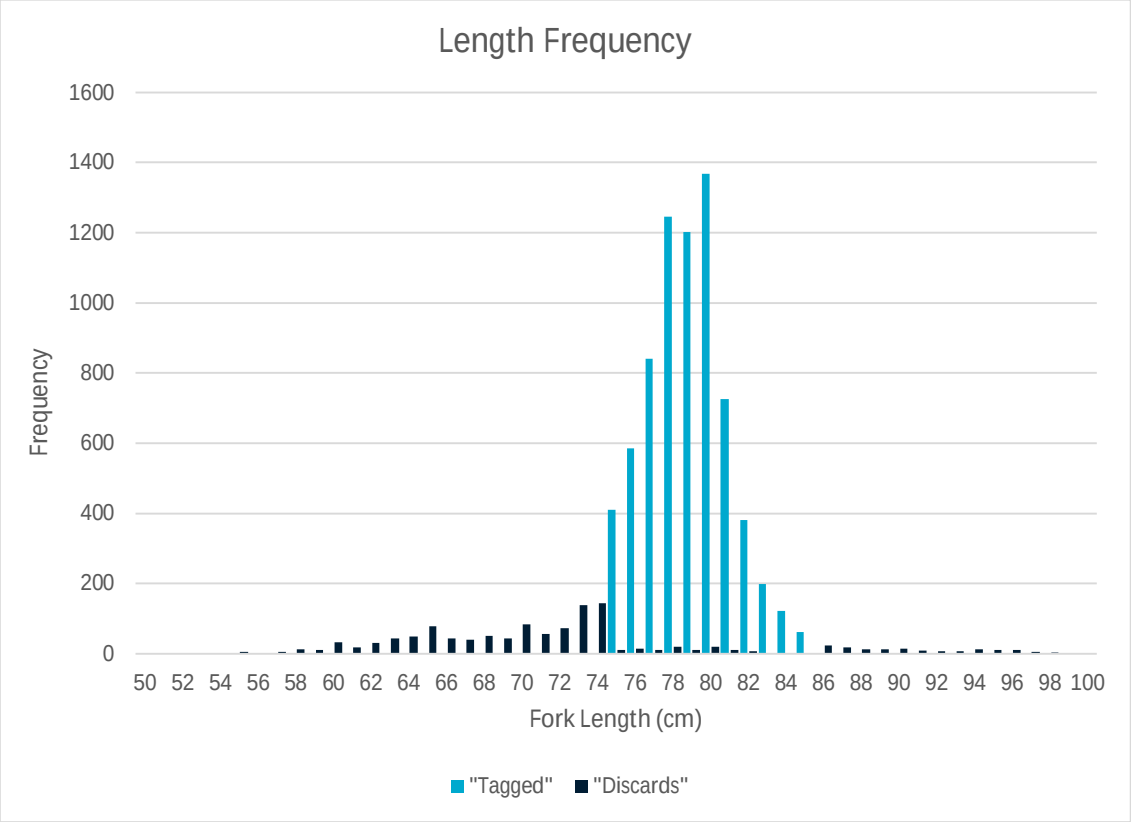

Figure 4 Size frequency for all SBT caught during Gene Tagging field work in 2021.

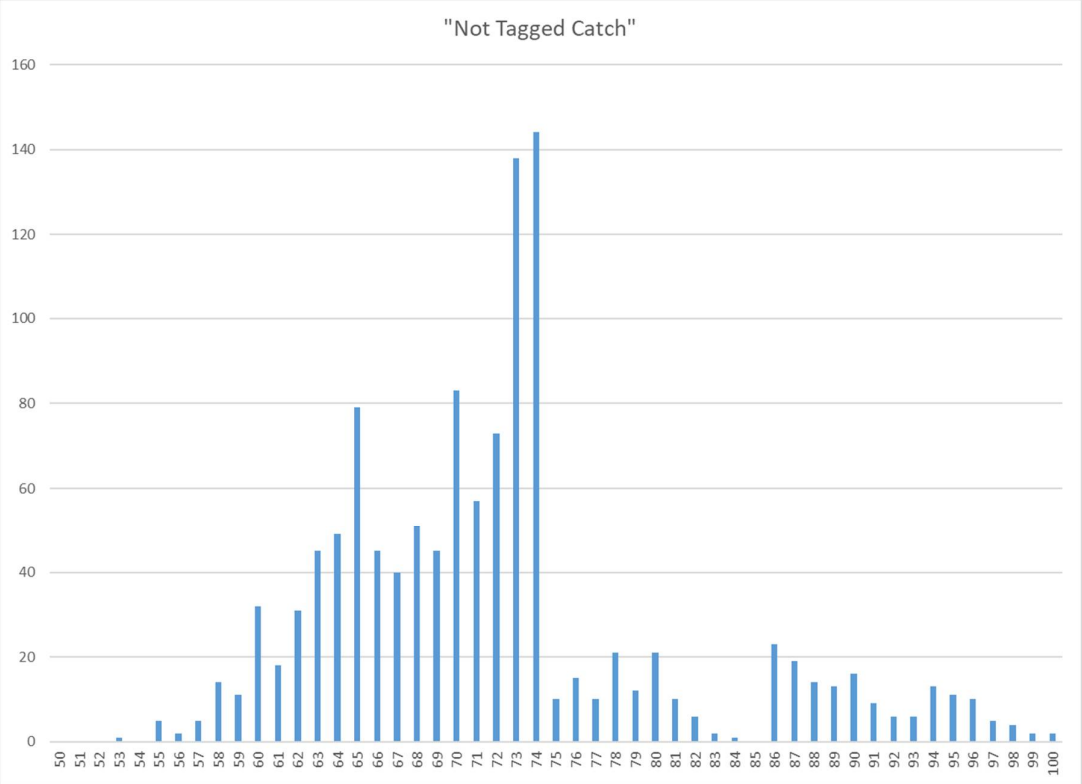

Figure 5 Size frequency for all SBT caught and released without tagging during Gene Tagging field work in 2021.

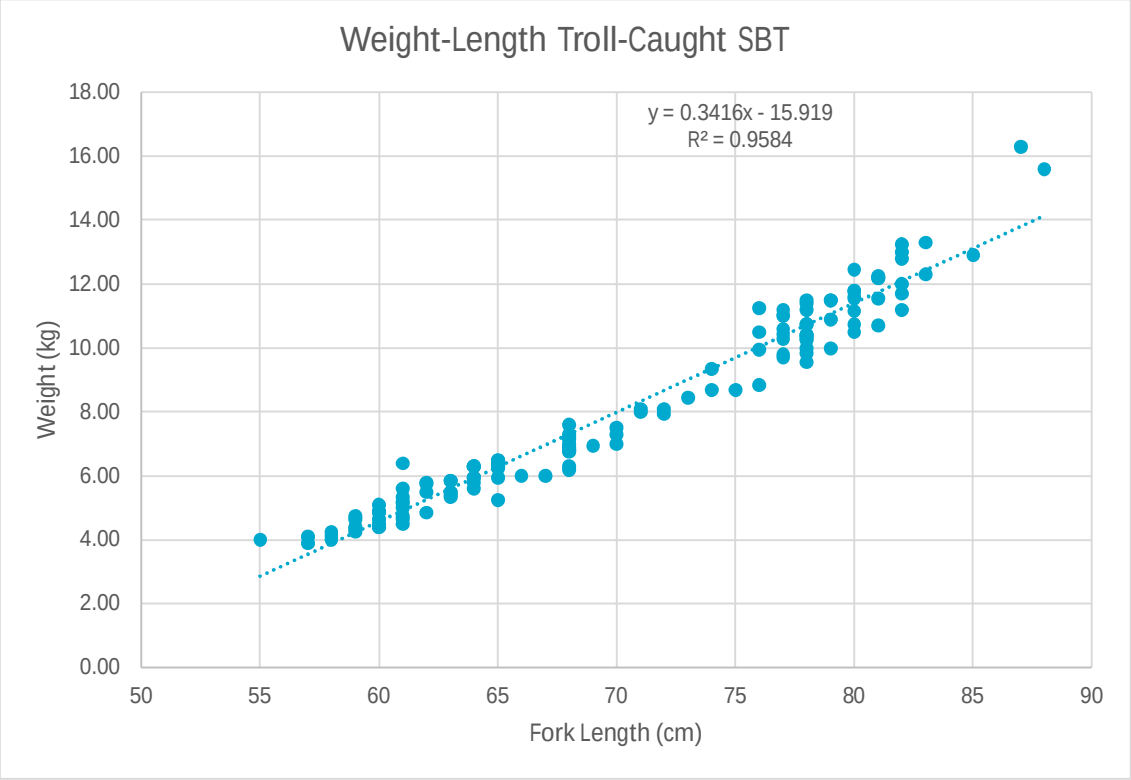

Figure 6 Fork Length-Weight relationship for troll-caught SBT during 2021 with trend line (Excel) and R-squared.

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