



Progress in stage 1 of gene-tagging 2017 and Research Mortality Allowance request for gene-tagging 2018

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Permit organisations:

- AFMA: Scientific Permit
- CSIRO Wildlife and Large Animal (CWLA) Animal Ethics Committee (AEC): Application No. 2016-18 Gene tagging southern Bluefin tuna
- South Australia's Director of Fisheries, PIRSA: S115 Ministerial Exemption ME9902885
- South Australian Department of Environment, Water and Natural Resources, Marine Parks Unit: Marine Parks Permit to undertake scientific research MR00099-2-V
- CCSBT Research Mortality Allowance

Abstract

The CCSBT gene-tagging recruitment monitoring program will provide an annual abundance estimate of juvenile SBT, from each year of tagging, for use in the SBT operating model and management procedure.

The gene-tagging program commenced a second year of tagging at sea in 2017. Nearly 8000 fish were tagged (via tissue biopsy) and released, during 20 days of sea-time in February-March 2017. A full trip report is provided. There were substantial improvements from 2017 based on experience from the pilot tagging work in 2017, and due to a different Master, vessel and more crew than the previous year.

For the 2018 gene-tagging program, a research mortality allowance of 3 tonnes is requested for gene-tagging in February-March 2018. The 2018 program will follow the specifications and sample sizes calculated in the design study (Preece et al, 2015).

1 Introduction

Gene-tagging is a recruitment monitoring method that has been proposed as an ongoing part of the CCSBT Scientific Research Program (Preece et al, 2015, 2014; Anon 2015, 2014). The program will provide an annual estimate of juvenile abundance, from each year of tagging, for use in the SBT operating model and future management procedure.

The CCSBT gene-tagging program commenced a second year of tagging at sea in 2017, following the pilot study in 2016 (Preece et al, 2017; Bradford et al, 2016). In 2017, 20 days of sea-time were available on a different vessel than the previous year, and with more crew. The trip report is available in Appendix 1.

A research mortality allowance of 3 tonnes is requested for the next gene-tagging field trip in February-March 2018. The 2018 program will follow the specifications and sample sizes calculated in the design study (Preece et al, 2015).

2 GT 2017 progress update

Stage 1 of the 2017 CCSBT gene-tagging program commenced with a successful tagging trip in February-March 2017. The field team “tagged” 7633 two-year-old fish by taking a small tissue biopsy. The tissue samples were collected over 20 days in the Great Australian Bight. Tagging operations commenced after completion of the commercial fishing season. Improvements in equipment were made based on experience from the pilot tagging work in 2016. A different Master, vessel, landing equipment and additional crew substantially contributed to the success of the 2017 field work. The target sample size was 5000 fish, but additional fish were collected to potentially increase the numbers of recaptures, to allow for failures in DNA extraction, and to allow for cohorts that are potentially larger than the design study anticipated. Details of the field trip are described in Appendix 1. The tissue samples have been safely archived at CSIRO.

The project requires a number of permits to proceed and we acknowledge the cooperation of the following agencies and committees: CSIRO Animal Ethics Committee, PIRSA Director of Fisheries, the South Australian Department of Environment, Water and Natural Resources’ Marine Parks Unit and AFMA.

Stage 2 of the project (2018) involves the collection of tissue samples from three-year-old fish from the surface fishery catch at time of harvest (June-August 2018). All tissue samples will be prepared for DNA extraction and, following appropriate quality control, sequencing. Analysis of the genotype data will be completed in late 2018 and an estimate of abundance of juveniles (aged 2 in 2017) will be available in early 2019, for use in a new Management Procedure.

3 Research Mortality Allowance used in 2017

Approximately 250 kg of the research mortality allowance (RMA) were used (from 30 fish mortalities) of the three tonnes allocated to the gene-tagging 2017 project (Appendix 1).

4 RMA request for gene-tagging in 2018

Project: Estimating absolute abundance of juvenile SBT from gene-tagging 2018

Principal Investigator: Ann Preece, CSIRO.

RMA timeframe: February 2018- March 2018

Research Mortality Allowance Request for 2017: 3 tonnes

Project aims and benefits:

The gene-tagging design study (Preece et al, 2015) recommended a sample size of 5000 for the initial tag and release component of the project. These fish are tagged by taking a small tissue sample and releasing the fish alive at sea. If more fish are encountered, in the right size class, additional samples (up to a total of 10,000) will be collected.

Three tonnes of RMA are requested for consideration by the Extended Scientific Committee and CCSBT for the at-sea tagging component of the 2018 GT recruitment monitoring program. The 2018 gene-tagging program is planned to commence in February-March 2018 for 20 days of tagging of small fish (70-85cm length) and will follow the protocols and procedures developed in previous years. Fish that are landed that are not in a suitable condition for tagging, or are outside the target length class, will be released without tagging or euthanized if injured.

The aim of the annual gene-tagging project is to provide estimates of absolute abundance of juvenile SBT for use in the SBT operating model and future management procedures.

5 References

Bradford R, Preece A and Davies C. (2016). Progress report on the implementation of the CCSBT gene-tagging pilot project in 2016. CCSBT-ESC/1609/07

Preece A, Eveson JP, Davies C, Grewe P, Hillary R and Bravington M. (2015) Report on gene-tagging design study. CCSBT-ESC/1509/18

Preece A, Bradford R, Grewe P, Eveson P, Farley J, Davies C. (2017). Progress report on the CCSBT pilot gene-tagging program in 2017. CCSBT-ESC/0917/07

Appendix 1: 2017 gene-tagging trip report by Russ Bradford

Southern Bluefin Tuna Gene Tagging – 2017 Field Work

In February 2016 CSIRO undertook a trial gene-tagging program for southern bluefin tuna (*Thunnus maccoyii*). Gene-tagging is a method that uses a small tissue sample from individuals to obtain a genetic signature unique for each individual fish caught and released in the field. A second suite of tissue samples is then collected during the annual fish harvest, with the genetic signatures being compared to the original data set to identify recaptured individuals. The field component focusses on fish within a narrow length range (70– 85 cm fork length) encompassing the main peak in the length distribution for age 2 fish. Harvest sampling focusses on age 3 fish (98-109cm) in order to identify individuals tagged in the previous year (age 2).

The field trial in 2016 identified several issues with the tissue sampling tools and charter arrangements which hampered the ability of the 2016 field work to meet the target number of fish to be tagged (5000). During 2016 CSIRO made a number of changes to the tools used to collect the tissue sample (gene tag). These included changes to the handle design to improve reliability, and changes to the design of the disposable tip to reduce variability in the amount of tissue collected as well as increase the overall amount of tissue collected.

Preparations for the 2017 field work season began in late 2016, with a start date for field work pencilled in for early March 2017 to coincide with the end of the SBT industry's purse seine fishery. However, fishing in the industry sector finished early and the gene tagging program was brought forward to start on 14 February 2017. Unfortunately the changes to the disposable tip were not able to be delivered via the commercial production company in time for the 2017 field work. None-the-less, the design changes were minor and could largely be replicated in the CSIRO workshop.

The FV *Yasmin* was chartered for field work in 2017; this vessel is part of the Australian Tuna Fishery fleet and was crewed by the Stehr Group. The crew totalled seven personnel, allowing for two crew to pole fish on-board for tagging. The FV *Yasmin* was well set out for the purpose with adequate deck space, live bait holding tanks and freezer space to store frozen bait and samples (Figure 1). An additional two bunks were available for CSIRO personnel.



Figure 1. Layout of the deck space on FV *Yasmin*.

Two CSIRO personnel arrived into Port Lincoln on 13 February 2017, aiming to set-up the tagging equipment early the following day. During the set-up, it was apparent that the tagging cradle that has been used for conventional tagging as well as gene tagging in 2016 was not suited to the vessel layout. Marcus Stehr (Australian Tuna Fisheries – Stehr Group) arranged for a chute (used for harvest operations) to be delivered to the vessel and attached (Figure 2). This was the only issue arising prior to the departure of the vessel.



Figure 2. Chute for tagging and measuring operations on FV *Yasmin*.

The vessel departed Port Lincoln at 1800 on 14 February, steaming directly to Rocky Island based on intelligence from a pre-voyage aerial survey. Fishing operations commenced on arrival at Rocky Island with the first tag being deployed at 0745; the last tag for the day was deployed at 1815, with approx. 1,100 tags deployed providing a flying start to the gene-tagging program. Field work was to be broken into two legs with a break after approx. 10 days to on-load additional bait, stores and change personnel (Master and one CSIRO tagger). Weather conditions deteriorated earlier than expected to bring the end of the first leg forward to 22 February 2017 (8 days). Change-over occurred in Streaky Bay with one day lost due to poor weather conditions. The second leg (11 days) of the program departed Streaky Bay on 23 February, and ended in Port Lincoln on 5 March 2017.

Fishing operations occurred at six main spots from Bell Point in the northwest, Fennalton Island, Cannan Reef (in the St Francis Archipelago), West of Ward, Rocky Island, to Wedge Island in the southeast, with the search area being bounded by -32.1338/131.8963 in the northwest and -35.3846/136.6534 in the southeast. The search area included the Fowlers Bay lumps, Nuyts Reef (including 9-mile and 12-mile lumps), Cabbage Patch, Baird Bay, and various lumps in the Flinders Island region (Figure 3).



Figure 3. Vessel track with main fishing regions annotated.

The majority of age 2 fish were located in a number of schools over multiple and non-consecutive days at Rocky Island and West of Ward, accounting for approx. 77% of all fish tagged (Table 1, Figure 4). The remaining age 2 fish were spread across three locations to the north of West of Ward, extending to Bell Point, and a small number (47) of age 2 fish tagged in the region of Wedge Island closer to Kangaroo Island in the southeast. Over the course of the field program three NSW Game Fishing external spaghetti tags were recovered. The tag numbers were noted and the fish released.

Table 1. Total fish tagged by location. Note that many areas were fished on non-consecutive days to allow for movement of fish through the region.

Location	Latitude	Longitude	Fish Tagged
Wedge Island	-35.2753	136.5576	47
Rocky Island	-34.8020	134.7287	3175
West of Ward	-33.6821	133.9823	2675
Cannan Reef	-32.6384	133.2444	451
Fennalton Island	-32.5993	133.2662	919
Bell Point	-32.3290	133.0258	366

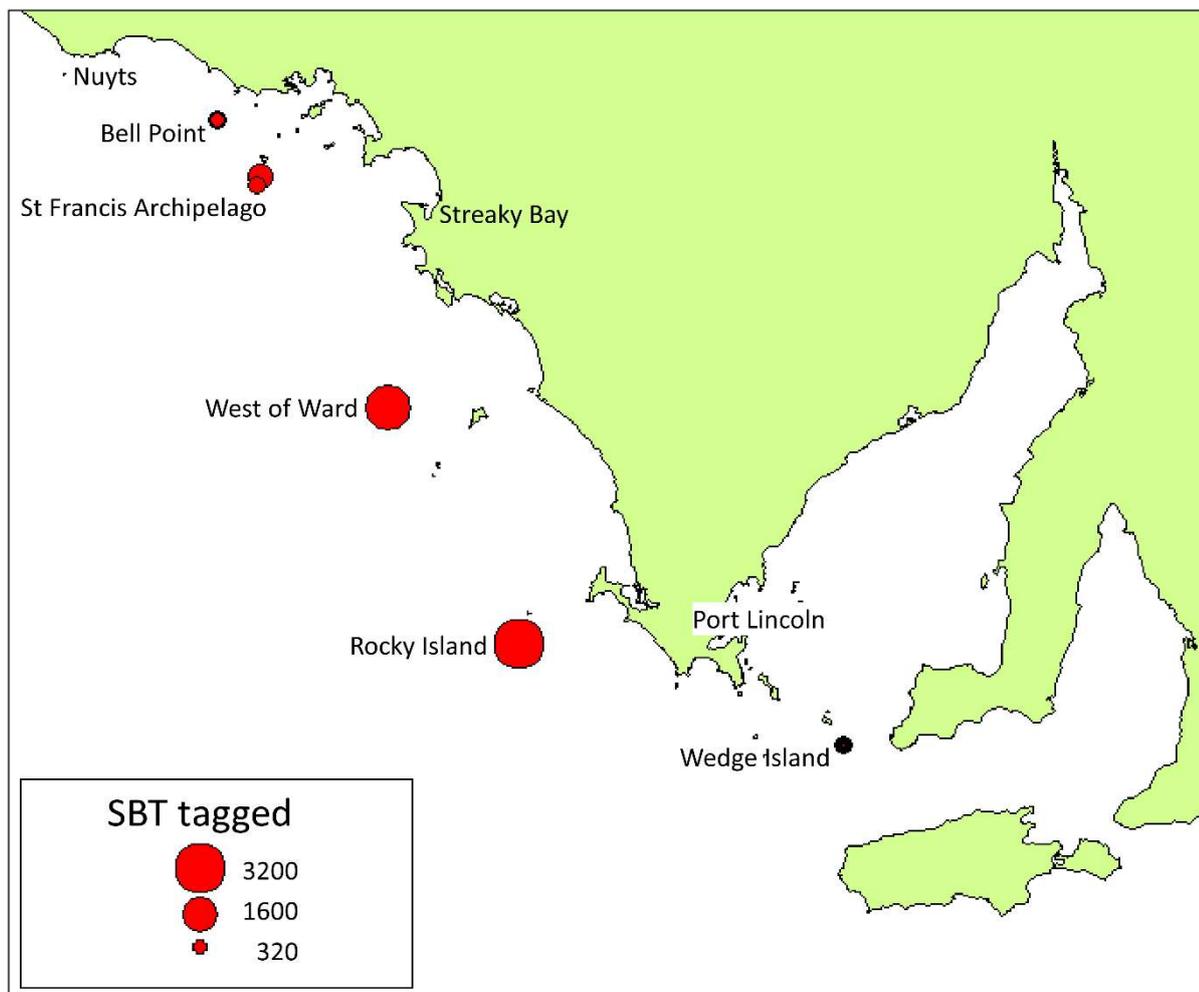


Figure 4. Main fishing regions with number of southern Bluefin tuna released at each site.

In total 7,633 age 2 SBT (Figure 5) were tagged over the course of the 2017 field work. A further 924 SBT were returned alive without tagging. These fish were either too small (i.e. ≤ 69 cm Fork Length), too large (i.e. >85 cm Fork Length), or had some minor damage that deemed them unsuitable for tagging. Minor damage included discolouration of the eye, a fish which had hit the deck of the vessel, a deep hook that took more than ~ 10 seconds to remove, existing damage unrelated to the poling process, or a slight gill bleed. All fish discarded in this process were considered of sufficient health to survive.

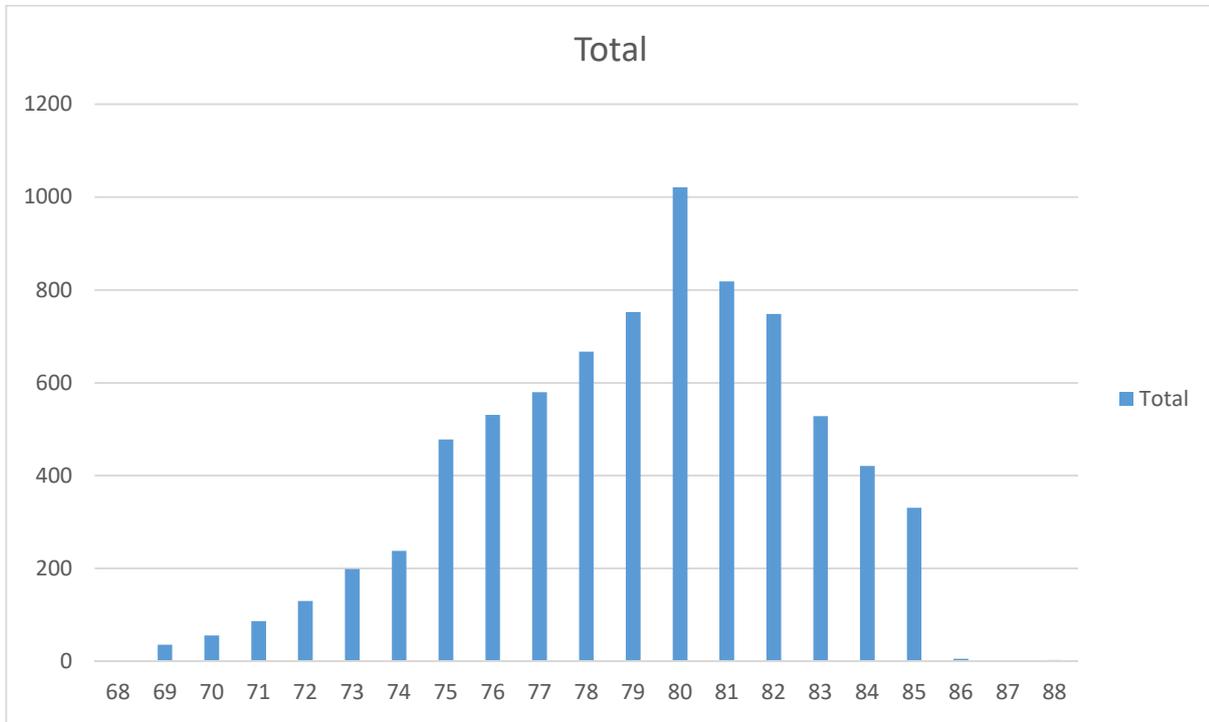


Figure 5. Length frequency diagram of tagged southern Bluefin tuna.

There were 30 SBT considered too damaged to survive a return to the water and these were killed for biological samples. Of the 30 killed, 28 were the result of gill damage from either the poling operation or the troll line that resulted in traumatic bleeding. The remaining two were the result of a broken caudal peduncle (poling damage) and a broken jaw (troll damage). Otoliths and muscle samples were collected from 25 of the mortalities (Figure 6).

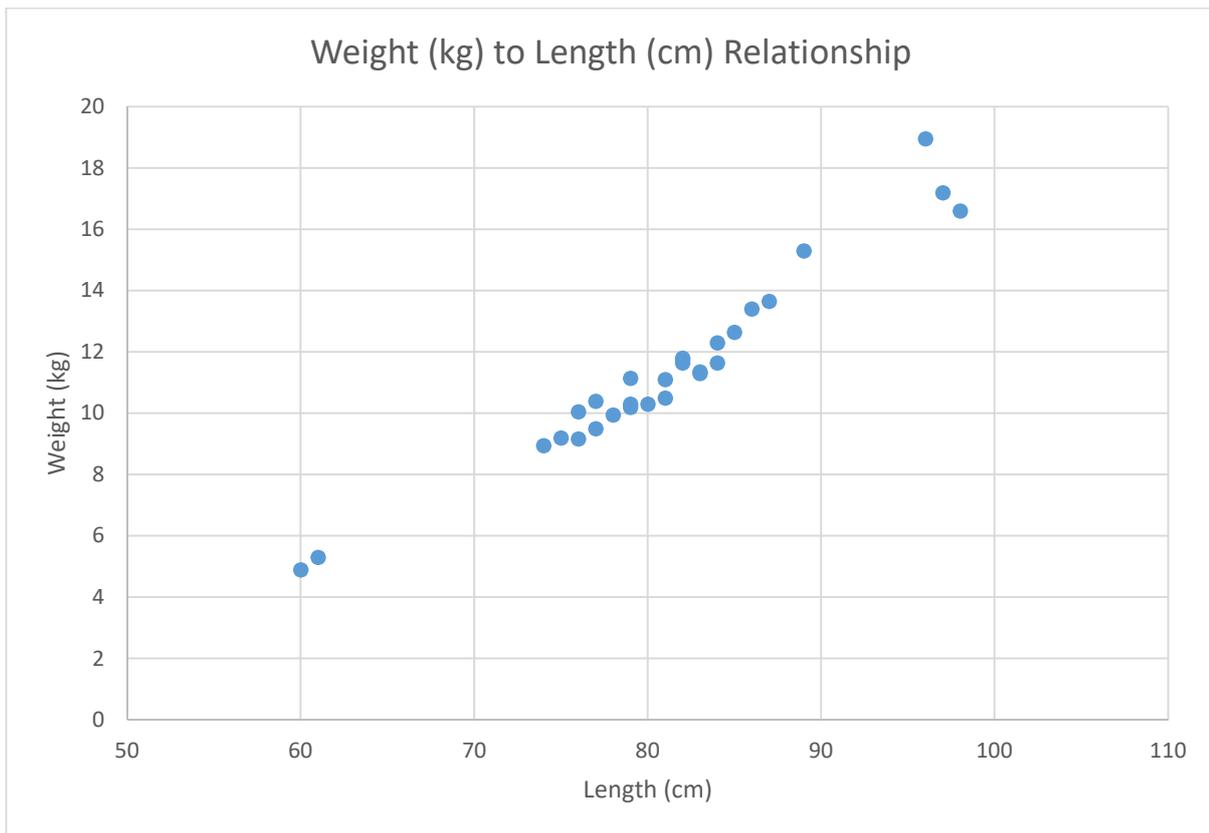


Figure 6. Weight - length relationship diagram of southern Bluefin tuna mortalities.

A number of improvements to procedures and tools were made during the course of this tagging program. Primary amongst them was the use of the chute to measure fish before obtaining the tissue sample and returning the fish to the water. The chute increased the speed with which fish could be presented to the taggers, reduced overall handling, and removed the requirement to carry fish. In previous tagging programs carrying fish resulted in many fish flapping out of the arms of the person carrying the fish and landing on the deck. Fish that hit the deck of the vessel are not used for tagging.

The number of SBT that hit the deck was minimal over the course of this field work program. The poling crew were also very experienced leading to few fish being damaged during the poling process. As a result the mortality rate in 2017 was greatly reduced from 47 in 2016 (~1.2% of tagged fish) to 30 in 2017 (~0.4% of tagged fish).

The number of crew on the vessel allowed for a crew member to assist the CSIRO tagger. The crew would bring the fish up to the measuring stop and call out the length of the fish. Fish outside of the required size range were immediately shunted through and returned to the water; a tissue sample was obtained from all other fish.

Further improvements could be made to improve the overall efficiency of the process. For example, the provision of an easily accessible freezer on the deck of the vessel would remove the need for entry to the galley (in wet weather gear). The chute could be fitted with a 'shower' for the fish on entry and additional sprinklers along its length to ensure fish arrive at the measuring stop clean, thus reducing the potential for DNA contamination between fish.

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