



Gene-tagging recruitment monitoring in 2018

Progress report and RMA request for gene-tagging in 2019

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Seatec for sample collection during harvesting.

Permit organisations:

- AFMA: Scientific Permit
- Animal Ethics Authority: CSIRO AEC 2016-18 Gene tagging southern Bluefin tuna
- Collection of biological material from Commonwealth Waters: AU-COM2017-388
- South Australia Marine Parks Permits: MR00099-3
- South Australia Ministerial Exemption (S115): ME9902955
- AFMA permits: 1003722
- 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 third year of tagging at sea in 2018. Nearly 8,200 fish were tagged (via tissue biopsy) and released during 20 days of sea-time in February-March 2018. A full trip report is provided in Appendix A. Only a small amount of the 2018 CCSBT research mortality allowance was used (39 mortalities, ~0.5t), thanks to the careful landing of fish by the crew of FV *Yasmin* and modifications made to the landing table and return chute. Biological samples were collected from these mortalities, including otoliths and vertebrae to provide age-length information.

The second year of harvest sampling in June and July 2018 has been completed with 15,000 tissue samples collected. The samples will be processed to extract the DNA which will then be sent for genotyping using the SNP makers developed by CSIRO. The full data set from fish tagged and released in 2017 and harvested in 2018 should be complete in late 2018. The abundance estimate will be provided through the CCSBT Scientific data exchange in May 2019.

For the 2019 gene-tagging program, a research mortality allowance of 3 tonnes is requested for gene-tagging in February-March 2019. The 2019 program will follow the specifications and sample sizes calculated in the design study (Preece et al., 2015).

1 Introduction

The SBT gene-tagging program aims to provide an absolute abundance estimate of juvenile SBT for use in stock assessments and management procedures. Gene-tagging is a method for recruitment monitoring of SBT that has been proposed and adopted as an ongoing part of the CCSBT Scientific Research Program (Preece et al., 2015, 2014, 2013; Anon 2015; Davies et al., 2007; Davies et al., 2008). Inclusion of these data in candidate management procedures is currently underway.

The gene-tagging methods were explored in a design study in 2015 (Preece et al., 2015). The pilot study tested the feasibility and logistics of collection of tissue samples 'at-sea' in 2016 and during the commercial harvest in on-shore processing facilities in 2017. The pilot study has produced the first estimate of absolute abundance of juvenile SBT (Preece et al., 2018). The abundance estimate is for the age-two cohort in the year the fish were tagged, i.e. an estimate of number of age-two fish in the population in 2016.

The CCSBT has agreed to continue the gene-tagging recruitment monitoring program to provide a time-series of abundance estimates. This report provides an update on progress from the 2017 and 2018 'at-sea' tagging; the collection of tissue samples 'at-harvest' in June-July, 2018; the CCSBT research mortality allowance (RMA) used in 2018; and, a request for RMA for the 2019 tagging program. A trip report for the 2018 tagging field work is provided in Appendix A.

2 Tagging progress update

The 2017 tagging program was conducted over 20 days in February-March, 2017 and results were reported to the CCSBT Extended Scientific Committee meeting in 2017 (Bradford and Preece, 2017). Over 7,600 fish were tagged and released alive. All 2017 gene tag tissue samples have been processed and sent for genotyping and will be compared with 2018 harvest samples when they are available (see below).

The 2018 tagging program was conducted from the 24th February to 16th March, 2018. Most of the commercial fishing operations had been completed prior to the tagging work commencing, and the few remaining commercial sets occurred away from the tagging areas outside of the Great Australian Bight. A total of 8,200 fish were tagged and released in 2018. Another 1,429 fish were released un-tagged because they were outside the target size range of 70-85cm. There were 39 mortalities from which biological samples were collected. Compared to 2017 there was a much more restricted spatial spread to the tagging in 2018, with most fish tagged in the eastern GAB area. Further west, fish were generally too small, and further east fish tended to be too big (see Appendix A).

The length frequency distribution of the fish caught in 2018 had two peaks, which may indicate that some age 1 fish were tagged (see figure 4 in Appendix A) in 2018. Otoliths and tails were collected from the mortalities and ageing of these may be able to provide more information on

whether some tagged fish should be excluded from the abundance analysis. There are more than 5000 tissue samples from fish longer than 74cm, which will be sufficient for the abundance analysis. We hope to provide more results from preliminary ageing work in early 2019. The tissue samples have been safely archived at CSIRO. Tagging data are managed in a database and will be provided to the CCSBT data exchange.

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, Australian Government Department of the Environment and Energy, and AFMA.

3 Harvest sampling in 2018

The second year of harvest sampling was conducted in 2018 between June and early August. The same procedures as used in 2017 were applied to ensure samples were collected throughout the season, and to minimise disruption to commercial operations. From early June 2018 through to 6th August, tails from fish in a target length class (98-109cm) were collected as the fish were processed in the on-shore processing facilities in Port Lincoln. These tails were marked to identify the length of the fish, and a small tissue sample was taken from each. We acknowledge the continued support and co-operation from the processing factories and their staff.

Tissue samples were collected from over 15,000 fish, and several hundred vertebrae were collected for ageing. Just over 12,000 tissue samples will be processed to extract DNA for genotyping. The additional 3,000 samples will be kept in reserve for later processing if required. For example, if the precision of the abundance estimate is lower than expected (few matches from larger than expected recruitment), or if there are poor genotyping results for a large number of samples.

Batches of the extracted DNA from these samples are progressively sent for genotyping by Diversity Array Technologies PTY LTD. A full set of data from the releases in 2017 and the harvest-samples in 2018 is planned to be complete in late 2018. From these data we will calculate an estimate of the number of aged-two fish in the population in 2017. This abundance estimate (the second in the series) will be available in early 2019 for submission to the CCSBT scientific data exchange and use in candidate MP development.

The vertebrae have been collected to provide annual estimates of the proportion of harvested fish aged 3 in each length bin (from 98-109cm) used in the recapture sampling. This work is in progress and will be used to adjust the calculation of the abundance estimate, if necessary. More information on these results will be reported in 2019.

4 RMA use in 2018 and request for 2019

There were 39 fish mortalities during the 2018 tagging field work, from which biological samples were collected, including otoliths and vertebrae for ageing. This is approximately 0.5t of the 3t research mortality allowance allocated for this project.

For the 2019 gene-tagging program, a research mortality allowance of 3t is requested for gene-tagging in February-March 2019. The 2019 program will follow the specifications and sample sizes calculated in the design study (Preece et al., 2015).

CCSBT RMA request

Project: Estimating absolute abundance of juvenile SBT from gene-tagging 2019

Principal Investigator: Ann Preece, CSIRO.

RMA timeframe: February - March 2019

Research Mortality Allowance Request for 2019: 3 tonnes

Project aims and benefits:

The gene-tagging design study (Preece et al., 2015) recommended a sample size of 5,000 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 2019 GT recruitment monitoring program. The 2019 gene-tagging program is planned to commence in February-March 2019 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 in candidate management procedures.

Appendix A Trip Report 2018. R Bradford.

A.1 CSIRO/CCSBT At-Sea Gene Tagging Field Work – 2018

The 2018 CSIRO/CCSBT Gene Tagging project's at-sea component was undertaken between 24 February and 16 March, 2018. Search effort was spread across the traditional range of the juvenile SBT from the Nuyts Archipelago (~132 °E) to a region south of the Althorpe Islands (136.9 °E) (Figure 1). Although southern Bluefin tuna were found throughout the search area, tag and release occurred over a much restricted range, with approx. 98% of SBT tagging occurring in the Rocky Island region and at Cabbage Patch. To the west (Nuyts Archipelago/St Francis) most SBT caught were below the target size range; while to the east (Gambier Islands: 136.42 °E) a large proportion of SBT caught were greater than the target size range.

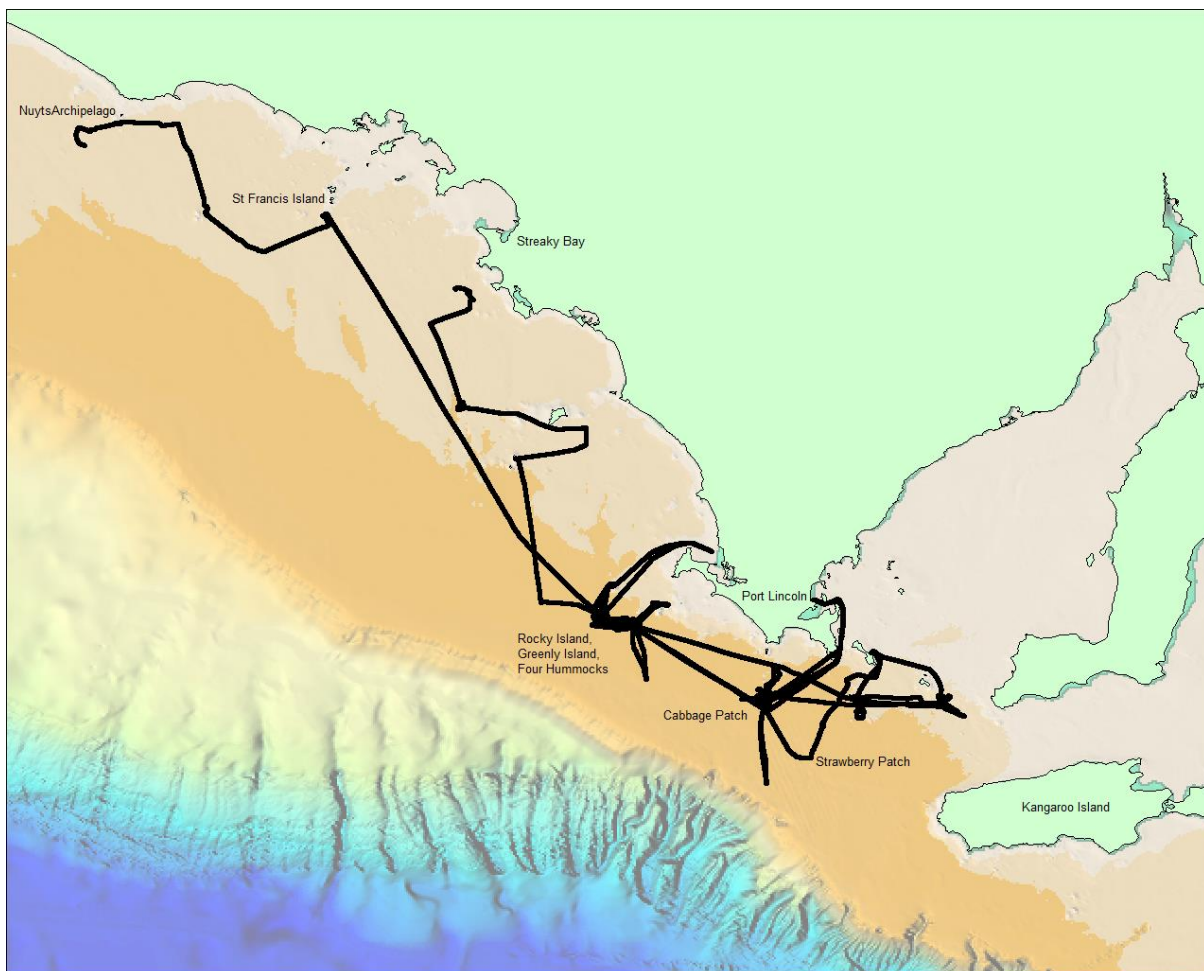


Figure 1: Track of FV Yasmin showing key fishing areas and extent of search effort.

In 2017, a chute was introduced as a refinement to our fishing practices and resulted in a reduction in the number of fish that landed on the deck of the vessel, rendering them unsuitable for tagging. In some cases, the fish that landed on the deck had to be killed due to the damage they sustained. In 2018, in partnership with the vessel charter company, we further refined the design of the chute. A new chute constructed of stainless steel and incorporating water jets was

built by the charter company (Figure 2). The chute was also custom designed to snugly attach to the landing table. The improved design has increased the efficiency of the procedure resulting in most fish landed being returned to the water in less time than last year.



Figure 2: Redesigned fish chute with water jets running.

In total 9,769 SBT were caught. Thirty-nine of those SBT were killed and biological samples retained. One hundred eleven (111) fish were captured on the troll lines, of which 10 were killed due to damage sustained (included in the above mortalities total). Of the remaining fish, 1,429 were outside of our target size range (70 – 85 cm) and immediately returned to the water; leaving 8,200 SBT that were gene-tagged before being returned to the water.

Mortalities ranged in length between 66 and 99 cm (fork length). Figure 3 provides the weight to length relationship for all mortalities.

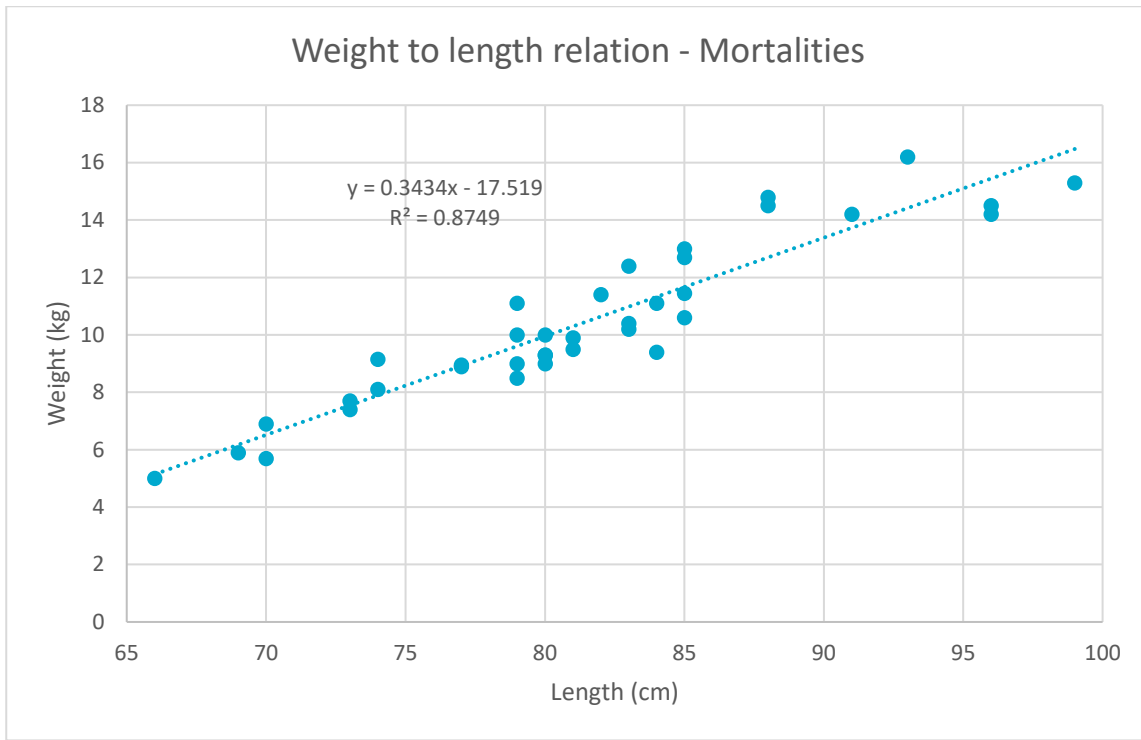


Figure 3: Weight-length relationship of southern Bluefin tuna killed during at-sea gene tagging in 2018.

The length frequency distribution of the tagged and discarded SBT illustrated two peaks in the fork length amongst the tagged fish (Figure 4). Otoliths and tail stocks have been collected from mortalities and will be examined for the age distribution within the two peaks. In total, 6, 852 SBT were collected within the second peak (75-85 cm FL).

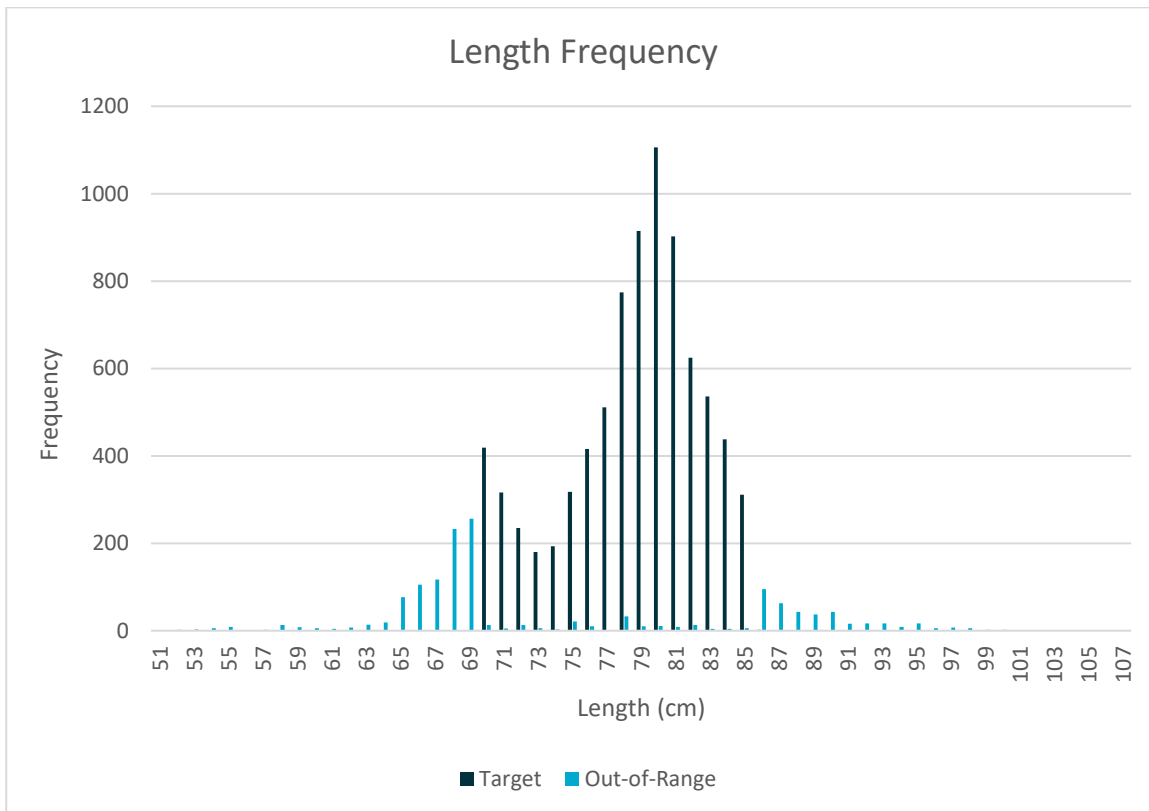


Figure 4: Length frequency of all southern Bluefin tuna gene tagged or returned alive without tagging.

Over the course of the 20 days at sea, several fish were caught that had been gene-tagged several days prior to the second capture. Figure 5 shows one such fish and indicates that the procedure results in very little external tissue damage and that healing appears to occur rapidly. Re-sampling some fish also provides an independent check on the DNA extraction and sequencing process.

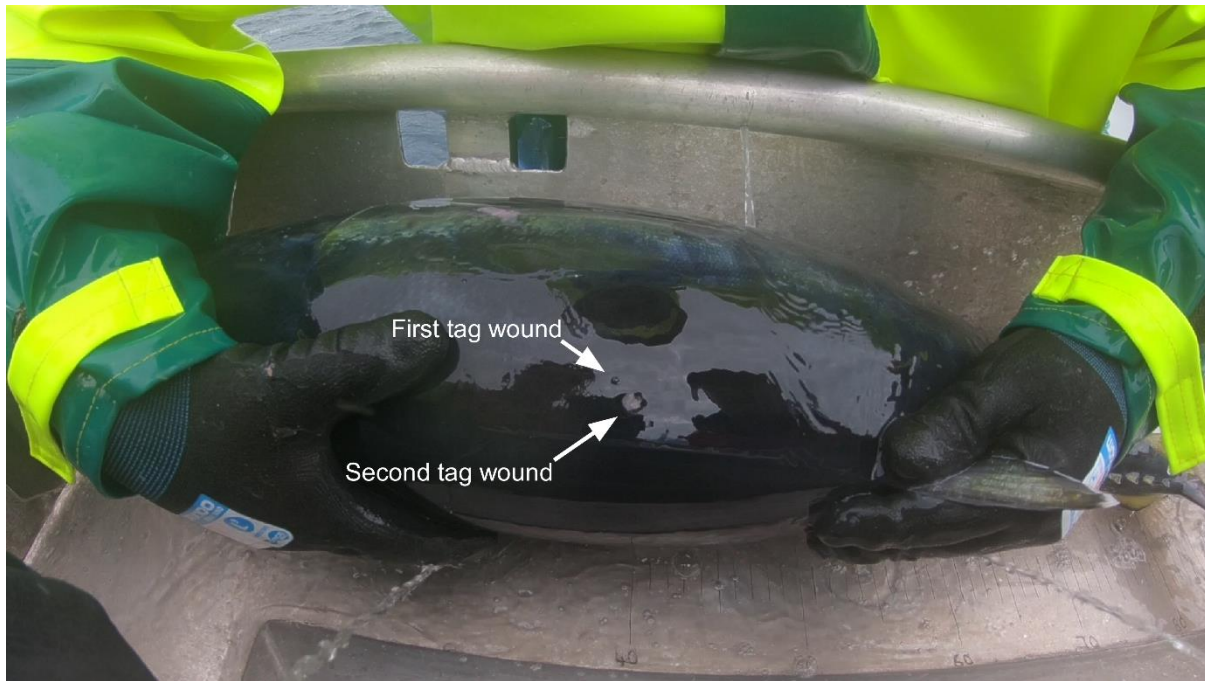


Figure 5: Recaptured southern Bluefin tuna showing the tissue damage resulting from taking a biopsy and the degree of healing occurring over a period of several days to a week.

A.2 Future Refinements and Repairs.

1. A number of refinements have been identified that should be incorporated into the protocols and equipment prior to gene tagging in 2019.
2. Although the refined chute & table were great improvements over previous years there remain some small modifications required to improve efficiency. The stern section of the landing table should have some additional padding, especially at the point where fish are entering the chute. With respect to the chute, there was some pooling of water at the entrance causing a lot of spray when a fish decided to flap. Cutting several holes or a small channel at the entrance should allow for excess water to drain away.
3. Currently the eski receiving samples is tied down onto the aft hatch. This hatch is slightly too low resulting in strain on the scribes back and legs (Figure 6). A custom bracket should be constructed that would allow for the eski to be tied down securely and be raised by 10 cm. If possible the bracket could have an extension arm (at the rear) to hold the data sheet securely (presently the scribe is required to hold the data sheets at all times).



Figure 6: Configuration of the eski on the aft deck hatch showing the posture of the scribe.

4. The pressure fit mechanism for the tip boxes requires repairs. The mechanism holding the boxes in place failed part-way through the trip and was replaced with a bolt system. Although this worked well, it requires some minor modifications to make replacing boxes quicker. The plate in which the box is fixed also requires some modification to account for the addition of some larger tip boxes in 2018.
5. The metal trays being used for vial storage work well for the empty vials. However, they take up excess space in the freezer once filled, and were being repacked into their original containers at the end of each day. During the latter stages of the 2018 trip we were putting the charged vials directly back into their original containers inside the eski. This should be carried through in future.
6. The rulers used to measure the mortalities and troll caught fish are now at the end of their useful life and should be replaced.
7. A vinyl cover for the motion-compensating scales would be a useful addition.
8. The use of PFDs (yolks and jackets) should be reviewed. Although in 2018 PFDs were used by CSIRO at all times when on the exposed deck space, there were times when the perceived risk was extremely low. It would be useful to have a ruling over when (e.g. under what sea conditions) and where PFDs are required for future trips.

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