

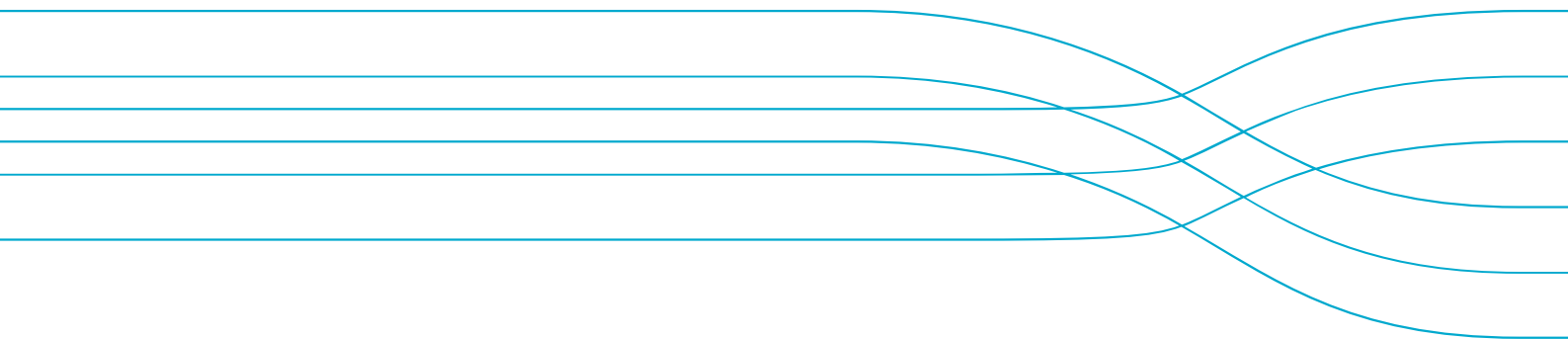


Progress report on the implementation of the CCSBT gene-tagging pilot project in 2016

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Abstract

The CCSBT agreed to commence a pilot gene-tagging project in 2016, as part of the CCSBT Scientific Research Program. Gene-tagging has been identified as an alternative to conventional tagging, with the research program designed to provide an estimate of absolute abundance of a juvenile cohort. If successful, the data collected from a long-term gene-tagging program are planned to be used in a new management procedure being developed for the CCSBT (Anon 2015). This report outlines the progress made in 2016 on the pilot gene-tagging project. Further work will occur in 2017 and early 2018, to complete the pilot study and provide the first estimate of abundance of 2 year old SBT.

The fieldwork for the pilot gene-tagging project commenced in February 2016, with an aim to tag (take tissue samples from) 5000 2 year old fish over 20 sea days. A total of 3,768 SBT were tagged. The tagging equipment worked well, with fish out of the water for a brief period of time (~20 seconds). Tagging using the gene-tagging tool was quicker and appears to be less invasive than conventional tagging or archival tagging methods.

Phase 1 of the pilot study did not reach the target number of fish (5000). However, the number tagged is sufficient to continue with the pilot tagging program as additional samples can be collected at the catch sampling stage to maintain a similar expected CV. Some preliminary genotyping has occurred and this will continue over the next months until all samples have been processed. Phase 2 involves taking tissue samples during harvest in June-August 2017 and initial scoping of the logistics for this stage have also been successfully completed. Further reporting on progress will be provided at the 2017 ESC.

1 Introduction

The CCSBT agreed to commence a pilot gene-tagging project in 2016, as part of the CCSBT Scientific Research Program. The aim of the pilot project is to test the feasibility and logistics of large-scale gene-tagging, as a method of recruitment monitoring (Preece et al. 2015). The pilot program will provide an abundance estimate of the age 2 cohort in 2016. If successful, the data from the long-term gene-tagging program are planned to be used in a new management procedure being developed for the CCSBT (Anon 2015). This report outlines the progress made in 2016 on the pilot gene-tagging project. Further work will occur in 2017 and early 2018, to complete the pilot study and provide the first estimate of abundance of 2-year old SBT.

Gene-tagging was first proposed at the CCSBT ESC in 2008 (Davies et al. 2008), however, it wasn't until the costs of processing and genotyping the tissue samples decreased sufficiently, that the method could be considered for routine monitoring. CSIRO developed a tissue sampling tool, the gene-tagging tool (GTT), to streamline tissue sample collection (Bradford et al. 2015). Coupled with recent developments in robotics and genotyping, the GTT has enabled cost reductions and sampling quality control for gene-tagging to be a cost-effective alternative to conventional tagging.

The design of the gene-tagging pilot study involves tagging age 2 SBT in the Great Australian Bight in 2016 and taking tissue samples from age 3 fish in the catch of surface fishery, at harvest, in 2017. The logistics of stage 1, the at-sea tagging and release of aged 2 fish, have been tested and the field trial results are summarised here.

2 Field Trials

The aim of the first phase of the pilot gene-tagging study was to test the logistics of tagging 5000 aged 2 fish in the Great Australian Bight, by taking a small tissue sample (using the GTT) from fish and releasing the fish alive. The target length range for the fish to tag was 70 to 85cm, which was based on all of the age-length data from otolith readings and is consistent with the size range of 2 year olds in conventional tagging data from the 1990s and 2000s. Field work followed the protocols developed for spatial distribution of tags for the 2001-2006 CCSBT conventional tagging program (Anon 2001), animal handling (CSIRO 2015), and specific protocols for taking and preserving high quality tissue samples for genetic analyses.

The field work was completed between 10 February and 02 March 2016 (see Appendix 1 for brief field trip log). Forecasted inclement weather resulted in the field trials being broken into two legs with a break of approximately 38 hours in Streaky Bay, South Australia. A total of 3,768 SBT were gene-tagged, averaging 188 fish per sea day. Mortalities totalled 47. The number of fish tagged is adequate for continuation into the catch sampling phase. To obtain the original target CV of the

abundance estimate, additional catch samples will be needed. These will be collected in 2017, if available, and will be processed if funds allow.

To ensure wide spatial distribution of releases, the tagging operations occurred over a large area and covered as many different schools of fish as possible. Commercial fishing operations were still underway in the south-eastern region of South Australia at the start of the pilot study. Therefore, gene-tagging operations were, for the most part, restricted to the north-western region to minimise the chances that tagged fish would be caught during the current fishing season. Gene-tagging extended to southern regions over the last 6-8 sea days after notification that commercial fishing had ceased (Fig. 1).



Figure 1. Vessel track with main fishing spots indicated.

Length, tissue sample number, date, time, location and tagger were recorded for each tissue sample collected. Gene tag samples were collected from fish ranging in length from 66 to 97 cm (mean = 78.86 ± 3.99 SE) (Fig. 2), slightly wider than the target length classes. The tissue samples that will be included in the final analysis can be selected from the full set. The 47 mortalities had

an average length of 77.66 cm (range: 60-95 cm). Length and weight was recorded for each mortality, but the calibration of the scales drifted through the voyage. A recalibration following the field work was used to reconstruct the weights (Fig. 3), however, these weights should be used with caution. Future field sampling should ensure the scales are kept in a location where there is little interference with ship operations that may result in drifting of the calibration. The weights of the mortalities averaged 11 kg (range: 4.9-19.2 kg). All mortalities were sampled for additional biological materials including otoliths. These otoliths may be used to supplement the length and direct age data used to create the age-length key for the SA surface fishery, and to fine-tune the target length class for age 2 fish.

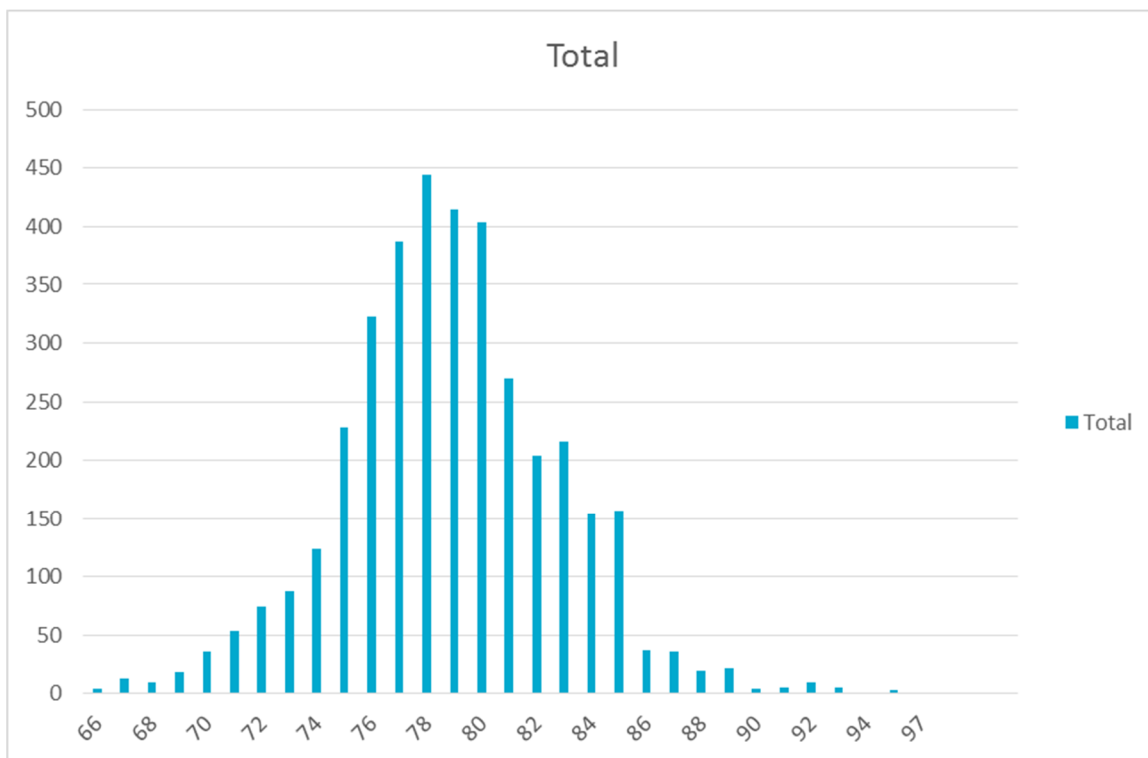


Figure 2. Length frequency distribution of gene tagged SBT.

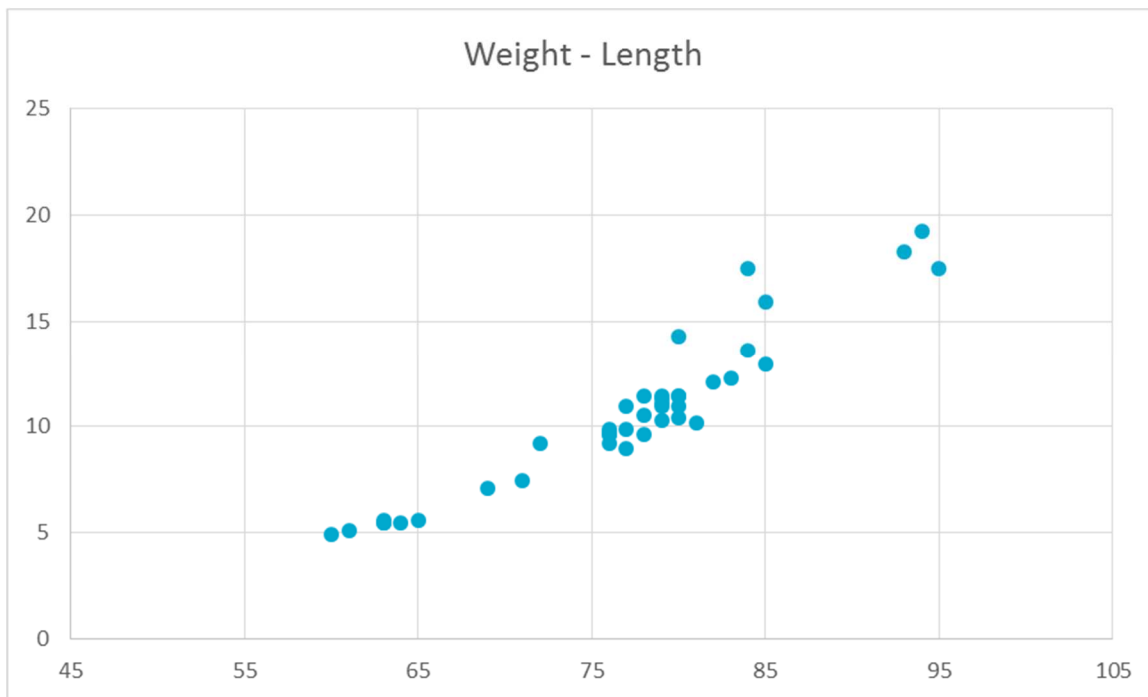


Figure 3. Weight-length relationship of SBT mortalities (after recalibration).

The gene-tagging equipment worked well, with fish out of the water for a very brief period of time, approximately 20 seconds. Tagging using the gene-tagging tool is quicker and appears to be less invasive than conventional tagging or archival tagging methods. The tagging tool takes a small amount of tissue (around the size of a grain of rice), and the tip is placed into a labelled tube and placed on ice immediately to preserve DNA quality.

The sparse distribution of SBT and adverse weather conditions made locating fish difficult at times. The aerial survey flew over the vessel on only 2 of the 20 days at sea, and the advice from the aerial survey team regarding location and sizes of fish was useful and accurate.

There was limited time after the 2015 Commission decision in which to commence gene-tagging and to tender for and charter a suitable fishing vessel. In future, the vessel should have a speed that will allow rapid transit between traditional fishing grounds, be equipped with a wider landing mattress, a breadth less than approx. 5-6 m, and a stable space for motion-compensated scales. The pilot field trials allowed the team to fine-tune improvements in the sampling equipment.

3 Next Steps

The specific genotyping method for processing the gene-tagging extracted DNA has been developed in a separate project funded by the CSIRO (Peter Grewe, Principle Investigator). Tests by Bradford et al. (2015) have shown that the tool developed by CSIRO obtained an uncontaminated tissue sample of adequate weight to provide sufficient DNA. The DNA has been extracted for the first 800 samples and the plates will soon be sent for genotyping, with the

remaining plates extracted and genotyped over the next year. Analysis to identify matching DNA profiles from the catch samples won't occur until late 2017 and will use data QC protocols and statistical routines developed by CSRIO.

The second stage of the pilot study is preparing for the catch sampling in June-August 2017. The original plan for the catch sampling phase was to collect 10,000 tissue samples at the time of harvest from the surface fishery from the grow-out operations in Port Lincoln. We are currently engaging the support and assistance from the South Australian Industry members to develop efficient methods for collecting these samples. Genotyping of all of the samples will not be completed until late 2017 or early 2018, and calculation of the initial estimate of abundance from the gene-tagging program is planned for early 2018.

Plans for the on-going monitoring of the juvenile population via a long-term gene-tagging program are described in Preece et al. (2016). The on-going gene-tagging program is proposed to commence tagging again in February 2017, pending confirmation of funding by the CCSBT. Field plans will be revised based on the pilot-study experience.

4 Summary

Phase 1 of the pilot gene-tagging study has successfully tested the logistics of at-sea tagging and use of the equipment developed for large-scale gene-tagging programs. A total 3,768 SBT were tagged during 20 days at sea, in February-March 2016.

The pilot study did not reach the target number of released fish (5000). However, the number tagged is large enough to continue with the pilot program and meet the target CV as additional samples can be collected at the catch sampling stage and processed if funds allow. Alternatively, the first abundance estimate may have a larger C.V. than planned. Initial DNA extractions and examination of DNA quality and quantity have been completed and this will continue over the coming months until all samples have been processed in preparation for large-scale genotyping. We will report further on progress on the genotyping and start of the pilot-study catch-sampling (June – August 2017) at the 2017 ESC.

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Appendix 1 Brief Field Trip Log

Field trials commenced on the 10th February 2016 from Port Lincoln, South Australia. The FV Celtic Rose (the chartered vessel) is a 22 m trawl vessel (Fig. 4), which was modified for pole and line fishing with the addition of a poling rack and landing bed (Fig. 5 & 6). No other modifications were made.



Figure 4: FV Celtic Rose (photo: J. Loughnan).



Figure 5: Single -person poling rack on port side of FV Celtic Rose for the capture of SBT (photo: R. Bradford).



Figure 6: Landing mattress immediately behind poling rack. Note the hessian cover was a late addition to try to reduce wear and tear on the foam mattress below. This modification did not work and was removed (photo: R. Bradford).

In order to familiarise the crew and taggers with the gene tagging procedures, on the 12th February we steamed to the lumps where on the previous day we observed a few isolated fish. Eight fish were caught and tagged before the return on effort was too low to justify remaining in

the area. A new patch of fish (Fig. 7) was located south-east of St Francis island late in the day, where a further 306 SBT were tagged providing an opportunity to resolve any issues and for minor adjustments to procedures to be made.



Figure 7: Patch of SBT near St Francis, South Australia (photo: R. Bradford).

Tagging operations continued in the north-western region around St Francis (Fig. 1) until late on the 18th February when we steamed to Streaky Bay due to forecasted extremely unfavourable weather conditions. Up to that point approx. 1690 SBT within the two-year old length [target] bracket had been tagged. This port time was used for a change of crew and a CSIRO tagger, as well as to repair equipment, confirm SBT distribution with the Aerial Survey's spotter pilot, and provide an update to CSIRO on tagging operations to date. Tagging operations were restricted to the north-western region in order to ensure there was no overlap in fishing operations with the commercial fleet which were still fishing in the south-east during that period.

The second leg began on 20th February with the vessel steaming towards St Francis and searching for SBT patches along the way. Information from the commercial fishing fleet indicated that commercial operations were still underway, hence our plan was to stay in the north-western region until further notice. Unfortunately the following two days were very unproductive with no fish located on the patches from the previous leg, possibly due to a dramatic change in water temperature (sea surface temperature had dropped approx. 2° C).

Tagging operations were shifted to the Nuyts archipelago and Yatala Reef, both regions of historically high catches and in the region of which recent spotting had confirmed SBT to be present. Over the course of the next two days approx. 780 SBT within the targeted length range were tagged. Many more SBT, primarily smaller than the targeted length range, were present within the patches. These smaller fish readily took the lure and resulted in lost time as the poler

either shook the fish off or the fish was landed and returned to the water on the opposite side of the vessel.

Commercial fishing operations were completed by 24th February. In order to spread the tagging effort throughout the region, over the remaining six charter days tagging operations moved progressively closer to Port Lincoln and target traditional SBT lumps along the way. A further 970 SBT were tagged during this period. However, unlike in the north-western region where SBT smaller than the targeted length made up a proportion of the catch, SBT larger than the targeted length range were encountered. These larger fish also appeared to quickly exclude 'target SBT' from the patch; at times, nine of ten poled fish were too large. The larger fish were more difficult to handle resulting in relatively more lost time per fish compared to the smaller fish encountered at Yatala Reef.

During the conventional tagging programs it was not uncommon to recapture a fish that had been tagged during the current season. It is important to note that although gene tagging was designed to be 'invisible', recently tagged fish could be recognised. On the 25th February two SBT were caught showing signs of previous gene tagging (Fig. 8). Both fish were caught within the same patch and separated by only a few poled fish. Figure 8 clearly shows the mark caused by most recent gene tag (identified by the trickle of blood, outlined with circle), with the previous mark anterior to the second mark (in square). The first gene tagging mark was healing well and showed no external indication of infection.



Figure 8. Previously gene tagged SBT showing the healing mark from the first tag (outlined by square) anterior to the re-tagging (outlined by circle).

Field trials finished with the vessel docking back in Port Lincoln on the 2nd March 2016. Over the course of the 20 charter days, the team tagged 3,768 SBT (averaging 188 fish per charter day).

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