



Results from the Pilot Gene-tagging Project

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Abstract

The southern bluefin tuna (SBT) pilot gene-tagging program commenced in 2016. The aims of the pilot study were to test the logistics and feasibility of gene-tagging SBT and to provide a fisheries-independent estimate of absolute abundance of juveniles. A total of 3,768 fish were tagged and released in 2016. The number of fish tagged did not meet the original target of 5000 fish, but it was possible to compensate for this by taking extra samples at harvest. A total of 16,490 tissue samples were collected during harvest, well in excess of the design study target of 10,000 samples. We acknowledge and thank the Australian SBT Industry members, factory Managers and staff, for access to their fish and facilities during the harvest.

Protocols were refined for DNA digestion, robotic extraction and quality controls. The extracted DNA is sequenced using specifically designed SNP markers. Not all samples had good quality or quantity of DNA and therefore not all samples were successfully sequenced. Fish with incomplete or poor genotype information (too few target SNP markers with good sequencing results) were excluded from the analysis. Approximately 74% of the tagged set and 86% of the harvest set were successfully sequenced and quality control to be included in the final analysis.

In total, 3456 fish were tagged (excluding fish with poor or failed genotyping), 15,391 fish were included in the harvest sample set, and 22 recaptures were detected. The abundance estimate is 2,417,786 with CV of the estimate of 0.21. The gene-tagging abundance estimate is close to the median estimate of age 2 fish in 2016 (2,102,853 fish aged 2 in 2016) from the 2017 stock assessment.

The pilot project has demonstrated the technical feasibility and logistics of a genetic tagging program for SBT and its potential to provide an absolute abundance estimate for monitoring and management purposes. The pilot SBT gene-tagging project has demonstrated collection of samples at sea and during commercial harvest from farms, collection of quality DNA, and high through-put processing from tissue to DNA and quality controlled genotypes. The CCSBT has commenced an on-going recruitment monitoring program using the gene-tagging method.

1 Background

The southern bluefin tuna (SBT) pilot gene-tagging program commenced in 2016. The aims of the pilot study were to test the logistics and feasibility of gene-tagging SBT and to provide a fisheries-independent estimate of absolute abundance of juveniles. The estimate of juvenile abundance will be used in the SBT operating models (OMs), future management procedures and to monitor rebuilding of the SBT stock. The experimental design, sample sizes, potential biases, costs and precision of estimates and examples of their use in stock assessment and management procedure models were explored in the 2015 CCSBT gene-tagging design study (Preece et al, 2015). Methods for incorporating these data in stock assessment models and a new management procedure were further explored and developed (Hillary et al., 2016 a&b). Simulation of these data has been included in the SBT projections code for Management Strategy Evaluation of candidate management procedures (Hillary et al., 2018a, b).

Gene-tagging of SBT involves 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 two sets of samples are genotyped and then compared in order to find the number of fish 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 tagging component of the project was completed in March 2016 (Bradford et al., 2016). In 2017, tissue sampling from 3-year-old SBT from the commercial harvest and DNA extraction and sequencing was completed. A preliminary summary of harvest sampling activities was provided to the Extended Scientific Committee in 2017 (Preece et al., 2017). The data analysis and calculation of an abundance estimate was completed in April 2018. These data were provided to the CCSBT as part of the scientific data exchange. Preliminary results were presented in a milestone report to the CCSBT in January 2018 and distributed to the Extended Scientific Committee inter-sessionally.

2 Method

The pilot project tested the methods, logistics and feasibility of the following steps:

1. Tag and release: Vessel charter and at-sea collection of tissue samples from age 2 fish in the Great Australian Bight during the summer of year 1 (2016).
2. Tissue collection during harvest: Collection of tissue sample from age 3 fish in year 2 (2017), during harvest of fish in farms which were caught by the Australian surface fishery.
3. DNA extraction and genotyping of tissue samples, using CSIRO SNP markers.

4. Data analysis and calculation of an abundance estimate. Provide the abundance estimate from the pilot gene-tagging program to the Extended Scientific committee for use in candidate Management Procedure and stock assessment models in 2018.

The design study noted extensions to the basic design, which should be considered after the initial logistics have been tested and demonstrated to be cost-effective in the pilot tagging study. These extensions included 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.

3 Results

3.1 Tag and release - tissue collection 2016

Results from the tagging component of the project were reported to the Extended Scientific Committee in 2016 (Bradford et al., 2016; Anon, 2016).

A total of 3,768 fish were tagged and released. Fish were tagged on 15 days in February/March 2016, across 14 locations, and from 37 schools. Some of the fish tagged were outside of the prescribed length range. All tagged fish have been included in the results presented here. The length range is being reviewed and is discussed further below. A total of 519 fish were released without tagging because they were either outside of the target size range, presented with minor damage, or were flapping too vigorously to safely tag. These released fish were considered to be in good to medium condition and likely to survive a return to the water. There were 47 SBT assessed to be damaged and unlikely to survive, and these were euthanized. Biological samples were collected from these mortalities, including 33 sets of otoliths. A full trip report was provided to the CCSBT ESC (Bradford et al., 2016).

A fish that appeared to have been tagged several days earlier was recaptured and tagged for a second time. A photo of the first wound was taken to show the extent of healing (see Bradford et al., 2016). The genotyping has confirmed a match within the set of releases.

The tagging component of the pilot study has demonstrated that it is possible to tag large numbers of fish by taking small tissue biopsies. The number of fish tagged did not meet the original target of 5000 fish, but as described in the design study (Preece et al. 2015), it is possible to compensate for this by taking extra samples at harvest. The CCSBT was advised that sufficient numbers had been tagged for the project to continue (Bradford et al., 2016).

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; quicker than conventional tagging methods, and considerably faster and less invasive than archival tagging methods. The gene-tagging tips allowed for collection of samples without any cross-contamination of DNA, which was a major successful outcome of this large-scale trial, confirming initial results from experimental trials in farm cages (Bradford et al., 2015).

3.2 Tissue collection during harvest in 2017

The method for collection of tissue samples during the commercial harvest from farms in Port Lincoln, South Australia, was developed during consultation with Industry representatives and members in 2016 and refined during the pilot project trials in 2017. Analysis of the size distributions of fish processed onshore and offshore indicated that there was no need to stratify sampling between these two modes of processing. Hence all sampling was conducted in the three participating onshore processing facilities. Harvest sampling was organised to collect tissue from as many fish as possible in the period available and within funding constraints, and to spread the collection across all the months of harvesting period to maximise sampling across cages, farms, Industry members and processing plants. Tissue samples were only collected from fish in the length range 98-109 cm. This range is assumed to represent 3 year-old fish (i.e. 3.5 years-old during the June-August harvest period).

We thank the Australian SBT Industry, Managers and staff in the processing factories for allowing CSIRO and Seatec access to their fish tails and facilities, and for assisting with trials of different methods as the techniques were refined.

A total of 16,490 tissue samples were collected, well in excess of the design study target of 10,000 samples.

Otoliths were collected from some harvested fish with known length to determine whether the length range being used for harvest sampling of 3 year olds is correct. In addition, vertebrae have been collected and a project to review vertebrae ageing methods (Gunn et al., 2008) is being considered. This will provide more information on impacts of uncertainties in age on the gene-tagging abundance estimates and allow for refinement of the length range used in sampling. A preliminary review of the available data, in preparation for the 2018 harvest sampling, indicated that the current length range did not need to be revised.

The harvest sampling component of the gene-tagging projects has demonstrated that it is feasible to collect large numbers of tissue samples from age 3 fish during processing after harvest in Port Lincoln in a cost effective manner.

3.3 DNA extraction and sequencing, using CSIRO SNP markers

The pilot gene-tagging project has processed over 20,000 tissue samples. Protocols were developed for DNA digestion, robotic extraction and quality controls. An archive plate of DNA is stored in -80 degree freezer at CSIRO. Data are recorded during all stages of the processing.

The extracted DNA is sequenced using specifically designed SNP markers. Each plate holds 92 gene-tagging samples, plus control samples. Not all samples had good quality or quantity of DNA and therefore not all samples were successfully sequenced.

The additional 6000 harvest samples were processed to extract the DNA. CSIRO provided additional funding to genotype these samples. These additional samples improved precision of the abundance estimate.

The pilot gene-tagging project has demonstrated efficient high throughput processing of thousands of tissue samples.

3.4 Data analysis and calculation of an abundance estimate

The genotype data were analysed to determine whether any individual fish were in both the tagged set and the harvest set (using the unique DNA fingerprint). This involved filtering the data to exclude fish with incomplete or poor genotype information (too few SNP markers with good sequencing results). Approximately 74% of the tagged set and 86% of the harvest set have been retained after filtering. The difference in the success rate between the tagged fish and the recaptured fish is possibly from: 1) the operating conditions at time of tissue collection (difficult conditions at sea versus a more controlled factory environment), and 2) the tip type (the tip design was refined in early 2017 in time for harvest tissue collection).

The final analysis identified 22 matches (recaptures) in the sub-sets of releases and harvest samples that remained after filtering. A fish was determined to have been recaptured if there was a fish with a matching set of markers in both data sets (releases and harvest samples sets).

In total, 3456 fish were tagged (excluding fish with poor or failed genotyping), 15391 fish were included in the harvest sample set, and 22 recaptures were detected. The abundance estimate is 2,417,786 with a CV of the estimate of 0.21. The median estimates of age 2 fish in 2016 from the 2017 stock assessment is 2,102,853. The additional harvest samples that were genotyped (~6000) increased the number of recaptures, and improved the precision of the abundance estimate from the pilot study.

4 Discussion

The pilot project has demonstrated the feasibility and logistics of a genetic tagging program for SBT and its potential to provide an absolute abundance estimate for monitoring and management purposes. This includes the successful collection of samples at sea and during commercial harvest from farms, collection of quality DNA, and high through-put processing from tissue to DNA and quality controlled genotypes. It was planned that 5000 tissue samples would be collected from fish tagged and released at sea, however bad weather resulted in 3768 tissue samples being collected in 2016. Additional tissue samples were collected during harvest sampling in 2017 to compensate, with 16,490 fish being sampled (well over the 10,000 samples initially planned). All tagging samples and harvest samples have been sequenced, where there was sufficient DNA quantity and quality. The flexibility to increase sample sizes at harvest to accommodate for lower than anticipated numbers of release samples, is a strong advantage of the mark-recapture estimator being used for gene-tagging (Preece et al., 2015). This level of adaptive sampling is not possible for conventional tagging programs as the recapture effort is controlled by the catch from the commercial sector and the comprehensiveness of the tag return program.

Quality control filtering of the sequencing data ensures only samples with good DNA are included in the analysis. This reduced the number of suitable samples for use in the abundance estimation. We have improved methods and protocols in the gene-tagging 2017 and 2018 projects to attempt to improve the percentage of samples that can be successfully genotyped.

The CCSBT has commenced an on-going recruitment monitoring program using the gene-tagging method. The 2017 tagging (Bradford et al, 2017) and 2018 tagging field work have been completed with over 8000 fish tagged in each field season. The 2018 harvest sampling is underway. In these subsequent years, improvements have been made based on experience and lessons learned from the pilot project. Tagging in 2017 used a refined sampling tip for tissue collection, and a new and more efficient charter vessel with a highly experienced fishing master and crew, and tagging in 2018 used the new “blue” tip which is mass produced on a mould (this was used for 2017 harvest sampling and had a high percentage of successful genotyping). Given the unpredictable nature of fishing, we recommend that the field team collect more tissue samples when possible, as the harvest sampling can be modified to collect fewer or more samples as needed to achieve the target coefficient of variation for the abundance estimate.

The abundance estimate from the pilot project is similar to estimates from the 2017 stock assessment, and will be used in candidate management procedures and future stock assessments. The final report of the pilot program will be presented at the 2018 ESC meeting. The gene-tagging project is a new and exciting addition to recruitment monitoring of SBT and has prospective applications for other species.

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