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Progress report on the CCSBT pilot gene-tagging program in 2017

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Contents

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Abstract

The CCSBT pilot gene-tagging program aims to test the feasibility and logistics of a large-scale mark-recapture program which uses DNA matching of tissue samples to estimate absolute abundance of juvenile SBT. The pilot program commenced in 2016 with the trial of at-sea tagging in February-March 2016. More than 3700 pole and line caught SBT were successfully biopsied and released. In 2017, “at-harvest” tissue collection was trialled, with over 15,000 samples collected (June-August 2017). The tagging samples from the 2016 pilot releases have undergone DNA extraction, and the extracted DNA has been sent to Diversity Arrays Technology (DArT) for sequencing. The at-harvest tissue samples will be processed and sequenced over the next few months to provide data for analysis and identification of matches (i.e., recaptures) from the pilot phase of the program. The abundance estimate from this program is intended to be used in the SBT operating models and management procedure. The pilot program is scheduled for completion in early 2018, with an abundance estimate available in time for the 2018 data exchange.

1 Introduction

The CCSBT pilot gene-tagging program commenced in 2016 as part of the CCSBT Scientific Research Program (Anon 2015; Bradford et al. 2016; Preece et al. 2015, 2014, 2013; Davies et al. 2007, 2008). The program has been designed to provide an estimate of absolute abundance of two-year-old fish in the year that they are "tagged and released" (Preece et al., 2015). The method involves matching of DNA to identify the same fish in two sets of tissue samples. The first set of samples is collected from two-year-old fish. The fish are caught, tagged (by taking a small tissue biopsy), and released alive to mix with untagged fish during their annual migration. The second set of samples is collected in the following year at time of harvest from three-year-old fish that have been caught in the Australian surface fishery. The biopsy tool has been developed by CSIRO for cost effective high quality tissue sampling (Bradford et al., 2015). All tissue samples are genotyped to provide a unique DNA fingerprint; based on the number of matches in DNA fingerprints between the two sets of samples (i.e., recaptures), an estimate of two-year-old abundance in the year the fish were "tagged" can be obtained.

The sample sizes for numbers of fish to tag and release, and numbers to sample from the catch, were optimised to find the minimum project costs, given that operations at sea are substantially more expensive than collection of tissue samples during harvest (Preece et al., 2014; 2015). The sample size estimates were based on recent estimates of juvenile cohort size from the 2014 stock assessment and the required precision (coefficient of variation) of the resultant abundance estimate, including effects of over-dispersion. The gene-tagging design study (Preece et al., 2015) examined potential sources of bias, methods for integrating the data from the gene-tagging program into the SBT operating models, and their potential use in future management procedures (see also Hillary et al., 2016a,b). The genetics and statistical analysis of sequencing data for genotyping have been developed under separate CSIRO research projects (Grewe and Eveson pers. comm.).

The aim of the pilot program is to test the feasibility and logistics of:

1. Collection of a large number of high quality tissue samples during tagging operations at sea.
2. Collection of tissue samples in large numbers at time of harvest in the Australian surface fishery in the Great Australian Bight.
3. High throughput (>15,000 samples/year) DNA extraction and sequencing.
4. Data analysis for genotyping, identification of recaptures and calculation of an abundance estimate with required precision for use in stock assessments and management procedures.

This report provides an update on progress of the pilot gene-tagging program. On-going recruitment monitoring using this method has been recommended by the ESC to provide an index of abundance for use in a new management procedure (Anon. 2015). A second year of gene-tagging in 2017 and plans for 2018 are reported on in Bradford and Preece (2017).

2 Tag and release – tissue collection

More than 3700 fish in the length class 70-85cm (corresponding to two-year-old fish) were tagged and released in the Great Australian Bight in 20 days of sea-time in February-March 2016. Tagging commenced after completion of the commercial surface fishery season, to avoid the risk of very short-term catches of tagged fish before they were able to mix with untagged fish in the population. The "tagging" involved taking a small (~15mg) tissue biopsy (the size of a grain of rice) using a specially designed tool (Bradford et al., 2015). No physical tag is required because DNA from the tissue sample provides an invisible, life-long "tag".

The pole and line capture and tissue sampling processes were based on the successful conventional tagging work undertaken in previous decades (Anon. 2001; Polacheck and Eveson, 2007). Date, tagger, fish length, fish condition, and injury data were also collected. Fish that exhibited any damage from pole and line landing, were listless or that had been out of the water for more than 30-40 seconds were not tagged. The poling rate was reduced to avoid landing fish before the tagger was ready. Biological samples were collected from a small number (n=47) of mortalities. The tagging speed in 2016 was around 20 seconds per fish, which is faster (i.e. fish are returned to the water more quickly) or similar to double tagging on SBT using conventional tags (~30 seconds) and substantially faster than archival tagging operations (several minutes).

The target number of releases was 5000 (Preece et al., 2015). Although the target number of releases was not achieved, the target precision of the abundance estimate can be still be achieved by collecting more samples at the time of harvest, which is an advantage of this form of mark-recapture estimator. Details of the pilot at-sea tagging, including a detailed trip report, were provided to the 2016 ESC (Bradford et al., 2016). The release stage of the pilot program successfully demonstrated that large numbers of tissue samples could be collected (see also Bradford and Preece, 2017).

3 At harvest - tissue collection

The fish tagged in 2016 are assumed to mix with the population of untagged two-year-old fish over the following 12 months. The design study examined this assumption and other potential biases that may affect the abundance estimates. Fish at age 3 return in large numbers to the Great Australian Bight and are caught by the Australian surface fishery each year (mainly in January-February) and placed in farms for a period of time before they are harvested and processed (June-August).

The pilot collection of tissue samples from three-year-olds during processing commenced in June 2017. The target number of three-year-old fish to collect samples from is 10,000. However, additional samples are being collected to improve the precision of the final abundance estimate, given the smaller than planned total number of releases, and to allow for selection of a subset of

samples if necessary. Considerable effort has gone into consultation with processing companies to refine the logistics and sensitivities of working within the processing operations, and to find an efficient and cost-effective method for tissue collection that does not interfere with the normal commercial processing. The ASBTIA members, their staff, the processing companies and staff, and Seatec have been generous and helpful in providing access to facilities and technical advice to assist in efficient, safe sample collection. Over 15,000 samples have been collected, and the logistics of the collection process has been modified and fine-tuned as a result of this experience. The pilot gene-tagging program has demonstrated that it is feasible to collect very large numbers of tissue samples at time of harvest.

4 DNA extraction and sequencing logistics

DNA extraction in the lab at CSIRO and sequencing at Diversity Array Technologies (DArT) has been completed for the release tissue samples collected in 2016. Some issues with DNA quantity were detected; the reasons for this have been addressed with a series of side experiments, leading to improved success rates for future sampling. Nevertheless, a large number of the 2016 samples appear to have sufficient DNA quantity and quality for successful application of the current sequencing methodology.

A set of specific markers (n=70 loci) and associated assays were developed in a separate CSIRO research study (Grewe pers. comm.), and the initial sets of samples processed using these assays have indicated that this represents a sufficient number of loci to uniquely identify individuals (Eveson pers. comm.). False positive matching rates are expected to be very low, with less than 1 fish in over 10^{10}. False negative rates will be identified in the data analysis phase. The final sequencing information will be further analysed in late 2017.

DNA extraction and sequencing of the at-harvest samples collected in 2017 has also commenced. This will be completed as quickly as possible to allow time for analysis and exchange of the first abundance estimate in 2018.

5 Analysis and related research

The analysis and genotyping phase to identify matches in the release and at-harvest samples will commence in late 2017. Identifying a matching DNA genotype in the two sample sets means that the same fish was tagged and released and then recaptured approximately 12 months later. The number of matches provides the data for calculating an estimate of abundance of two-year-old fish in the year they were tagged (2016), which we anticipate will be available in early 2018. A full report on the completed Gene-Tagging Pilot program will be completed by mid-2018.

The size ranges for sample collection for both two- and three-year-old fish are based on the available data from previous tagging programs, direct age and length data, and results from previous growth studies (Eveson et al., 2004), adjusted for the time of sampling. Further information will be available from otoliths collected from the mortalities during tagging operations, and from fish filleted in the processing factories, to account for uncertainty in length-at-age of the particular cohort at the time of tagging and harvest. The potential use of vertebrae as another source of length-at-age data is also being explored. Accounting for uncertainty in the age of releases and recaptures in the likelihood function for the operating model and projections model (when simulating these data for testing candidate management procedures) has been considered in Hillary et al. (2016a) and will be addressed in the analysis phase of the project.

The proposed mark-recapture estimation model assumes that tagging mortality is very small. Considerable effort is focussed on minimising release mortality. In particular, fish are not biopsied and released if there is any visible damage, if the fish appears to have landed badly, or if the fish is not in a vigorous condition (as per the tagging protocols; Bradford et al., 2009). Fish condition and tagger are recorded and these data will form part of the analysis. In the case of the SBT conventional mark-recapture program, any releases with fish injury recorded as more than slight were omitted from the analyses to avoid potential biases due to tag-related mortality (Polacheck and Eveson, 2007). Tag-related mortality for SBT was examined in the tag-seeding experiments conducted in the farms, and there was no evidence of higher mortality rates for tagged fish (Stanley and Polacheck, 2003). Experiments using the gene-tagging tool used to take the small biopsy also indicated that there was no tag-related mortality from this method, and that wounds were healed within three weeks and were invisible within 73 days (Bradford et al., 2015). While these results do not exclude the potential for tag-related mortality, it does suggest that the effect size is likely to be small.

Work has progressed on the methods for inclusion of the gene-tagging abundance estimate in the operating model and potential use in new management procedures (Hillary et al., 2016b). This was originally addressed in the gene-tagging design study which demonstrated the proposed methods using conventional tag data (Preece et al., 2015). The gene-tagging program anticipates providing annual estimates of two-year-old abundance for 2016 onwards (with an estimate for 2016 available in 2018) for inclusion in reconditioning the operating models for management strategy evaluation of candidate management procedures.

6 Conclusion

The pilot gene-tagging program is progressing successfully and is approaching analysis stage, for provision of an absolute abundance estimate of juvenile (two-year-old) SBT.

The feasibility and logistics of collecting large numbers of tissue samples at sea and at harvest has been tested, with collection of over 3700 samples at sea in 2016 and over 15,000 samples at harvest in 2017.

DNA has been extracted from the majority of samples processed to date, with sufficient quality and quantity for DNA sequencing. The DNA loci detected by the specialised primer assays developed by CSIRO are sufficient to match a fish to itself with less than 1 in 10^{10} rate of false positive matches. False negatives can be detected and checked in the data analysis phase.

The data analysis for genotyping and calculation of an abundance estimate with reasonable precision for use in stock assessments and management procedures will commence after complete sequencing of all available samples. The abundance estimate should be available in early 2018, in time for inclusion in the next reconditioning of the SBT operating models which will be used for management strategy evaluation of the new candidate management procedures.

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