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Update on the SBT close-kin tissue sampling, processing and kin-finding 2023

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Abstract

Muscle tissue samples collected from adult southern bluefin tuna (SBT) landed by the Indonesian longline fishery in 2019/20 and 2020/21 were transported to Australia in late 2022. The tissue was subsampled, DNA extracted, and sequenced. The kin-finding analysis to identify parent-offspring pairs (POPs) was updated to include the new Indonesian adult data. The results were provided to the CCSBT in May 2023 and are used in the 2023 stock assessment. A total of 120 POPs have now been identified. The half-sibling pairs (HSP) analysis was not updated because there were no new juvenile data; the juveniles sampled in 2020 and 2021 were sequenced and had been included in the HSP analysis in 2022.

Muscle tissue collected from juvenile SBT in Australia in 2022 will be sequenced later this year and the data will be included in kin-finding analyses in 2024. Sampling of juvenile SBT in 2023 is complete and the samples will be transported to Hobart soon.

As reported last year (ESC27_09_CCSBT_CKMR) muscle tissue samples were not collected from the Indonesian longline fishery in 2021/22 due to disruptions caused by Institutional changes in Indonesia. In January this year, we held a training workshop in Benoa for Enumerators from the Directorate General of Capture Fisheries (DGCF) on how to collect SBT muscle tissue and otolith samples, to enable the SBT monitoring and sampling in Benoa to recommence. Sampling recommenced in February and 148 muscle tissue samples were collected. We anticipate sampling will recommence in September for the 2023/24 spawning season, with the aim to sample 1500 SBT as per previous years, and we propose to collect an additional muscle tissue samples in Indonesia in 2023/24 to compensate for the lack of Indonesian muscle tissue sampling in the previous two seasons.

Introduction

In 2013, the Extended Scientific Committee (ESC) developed a new Scientific Research Plan (SRP) for southern bluefin tuna (SBT). The specific projects and priorities for the SRP were considered in both 2014 and 2015. High priority items identified in the work plan included the continued collection and genotyping of tissue samples for close-kin mark-recapture (CKMR) genetics to assess the abundance of adult SBT. The CCSBT has funded the collection and archiving of SBT muscle tissue since 2014/15, and DNA extraction and sequencing of these tissue samples since 2015/16. These samples and data have subsequently contributed to the completion of a second CKMR abundance estimation project that incorporated both parent-offspring pairs (POPs) and half-sibling pairs (HSPs), which was reported to the ESC in 2018 (Davies et al. 2018; 2020). Since 2018, the CCSBT has also funded the analysis of sequencing data to find POPs and HSPs (close-kin identification) on an annual basis. Table 1 shows the work completed by spawning season since 2014/15. In 2019, the CCSBT agreed to increase the number of tissue samples genotyped from ~2000 to 3100 annually (i.e., the total number of adults and juveniles collected annually) to increase the number of “POPs per cohort comparison” (Anon 2019). In this paper we provide an update on progress of activities in 2023.

Table 1. Summary of SBT close-kin work undertaken as part of CCSBT projects since 2015.

Sampling season	No samples collected Adults/Juveniles	Genotyping completed	Kin-finding completed
2014/15	1500/1600	Yes	Yes
2015/16	1500/1600	Yes	Yes
2016/17	1500/1600	Yes	Yes
2017/18	1500/1600	Yes	Yes
2018/19	1500/1600	Yes	Yes
2019/20	1500/1600	Yes	Yes
2020/21	1500/1600	Yes	Yes
2021/22	0/1600	Planned for late 2023 (juveniles only as no adults were sampled)	Planned for 2024
2022/23	148/1600	Planned for 2024 (juveniles and a small number of adults)	Planned for 2025

Muscle tissue collection

Targeted sampling of adult SBT was reduced at the Benoa Fishing Port in Indonesia in the 2022/23 spawning season due to disruptions caused by institutional changes in Indonesia. In January, a training workshop was held for DGCF Enumerators in Benoa on how to collect SBT muscle tissue and otolith samples to enable the monitoring and sampling in Benoa to recommence (Figure 1). Sampling recommenced in February and 148 muscle tissue samples were collected. We anticipate full sampling will recommence in September for the 2023/24 spawning season (1500 samples) and we propose to collect an additional muscle tissue samples to compensate for the lack of Indonesian muscle tissue sampling in the previous two seasons.

The total for the targeted sampling of juveniles in Australia was met in 2023 with 1600 samples collected at the tuna processors during harvest operations in Port Lincoln, South Australia, in June-July. Tissue was obtained from fish ranging from 89-109 cm fork length (FL) to cover the size range of 3-year-olds (see Preece et al. 2023). The tissue samples were placed in 2ml tubes and frozen according to protocols provided by CSIRO and will be transported to Hobart. The frozen muscle tissue samples are stored in consecutively labelled boxes with 100 positions (10 by 10) in each box (A01 through J10). Individual samples are given a unique identification label (e.g., SbPL2014_Bx01_A01).

Figure 1. Training workshop was held for DGCF Enumerators in Benoa on how to collect SBT muscle tissue and otolith samples.

DNA extraction and sequencing

Muscle tissue samples from adults collected in Indonesia in 2019/20 and 2020/21 were subsampled and DNA extracted. The DNA was extracted using a magnetic bead-based extraction protocol (Machery Nagel Nucleomag) kit on an Eppendorf EP motion robot to produce a 90uL archive and 30uL working stock of DNA in micro-titre format plates. Working stock plates of extracted DNA were shipped to Diversity Arrays Technology (DArT) in Canberra for sequencing (referred to as “DArTcap”) of approximately 2000 single nucleotide polymorphic loci (SNPs). Archive plates of extracted DNA are stored in a dedicated -80°C freezer located at CSIRO Hobart. All sequencing data (2 years) were sent to CSIRO Hobart in early 2023 for inclusion in the kin-finding (below). There were no new juvenile data because juveniles sampled in 2020 and 2021 were sequenced and included in kin-finding last year.

Kin-finding

The kin-finding analysis database used for identification of POPs and HSPs was updated to include the DArT sequencing data for adult samples collected in 2019/20 and 2020/21.

Prior to kin-finding, the sequencing data are used to “call the genotype” for each fish and locus in the data (i.e., to infer the pair of alleles present). This genotype-calling entails complicated algorithms developed by CSIRO specifically for DArTcap sequencing data and estimates the genotyping error rates for each locus, which is important in the identification of HSPs. A plate-level standardization was applied to the sequence count data from all years before calling the genotypes (Farley et al. 2019). This ensured that, for a given loci, the average count across all samples on a plate was the same for each plate.

A series of quality control (QC) steps were applied to the genotyped data to remove fish with unreliable genotype calls. These include:

- a test for heterogeneity to remove fish with an unexpectedly high number of heterozygous loci, which could be an indication of cross-contamination of DNA between individuals;
- a test of whether an individual's genotype could plausibly have been drawn from the 'stock' represented by the rest of the samples to remove fish potentially mis-identified as SBT; and
- a test for an over-representation of null alleles in each individual genotype to remove degraded samples.

After applying the QC steps, 11,261 adults and 18,157 juveniles remained for kin-finding (Table 2).

POP-finding

We used the genotype data to identify POPs using the same method as previous years, which is a modified Mendelian-exclusion statistic referred to as the Weighted-PSeudo-EXclusion (WPSEX) statistic (see Appendix B of Bravington et al. 2017).

Figure 2 shows part of the histogram of the WPSEX statistic comparing across all possible genotyped adult-juvenile pairs (18,157 juveniles x 11,261 adults = 204.5 million comparisons). The POPs are visible as a small bump to the left of the dashed blue line. Most of the histogram (to the right) has been truncated, because otherwise the POPs are too few compared to the gigantic bump of unrelated pairs (the peak of which is around 0.116, where theory predicts it should be based on allele frequencies of each locus) and could not be visualized. The giant bump of unrelated pairs drops off very quickly to the left of ~0.075, and the “tail” between ~0.045-0.075 will contain a number of adult/juvenile HSPs and grandparent-grandoffspring pairs. The gap between this “tail” and the POPs bump has decreased over the years, which is to be expected given the number of comparisons has increased exponentially. In future, additional analyses may be required to help determine whether pairs in this uncertain zone between ~0.04-0.05 are POPs or less related pairs.

The number of POPs identified in this data set is 77. Including the POPs that were identified previously using microsatellites (recall that the genotyping method changed after 2015 from using

microsatellites to DArTcap sequencing; see Bravington et al. 2015; 2017), we now have a total of 120 pairs. The breakdown by juvenile birth year and adult capture year is given in Table 3.

Table 2. Number of fish used in the kin-finding analyses this year after quality control checks were applied. For the adults, samples were collected from Indonesia in the fishing season ending in the year shown (i.e., samples collected over the 2005/06 fishing season are referred to as year 2006).

Year	Adults	Juveniles
2006	0	1317
2007	0	1325
2008	0	1356
2009	0	1347
2010	972	1315
2011	958	963
2012	536	876
2013	959	903
2014	922	899
2015	0	953
2016	951	854
2017	971	948
2018	700	756
2019	1440	1449
2020	1421	1512
2021	1431	1384
Total	**11261**	**18157**

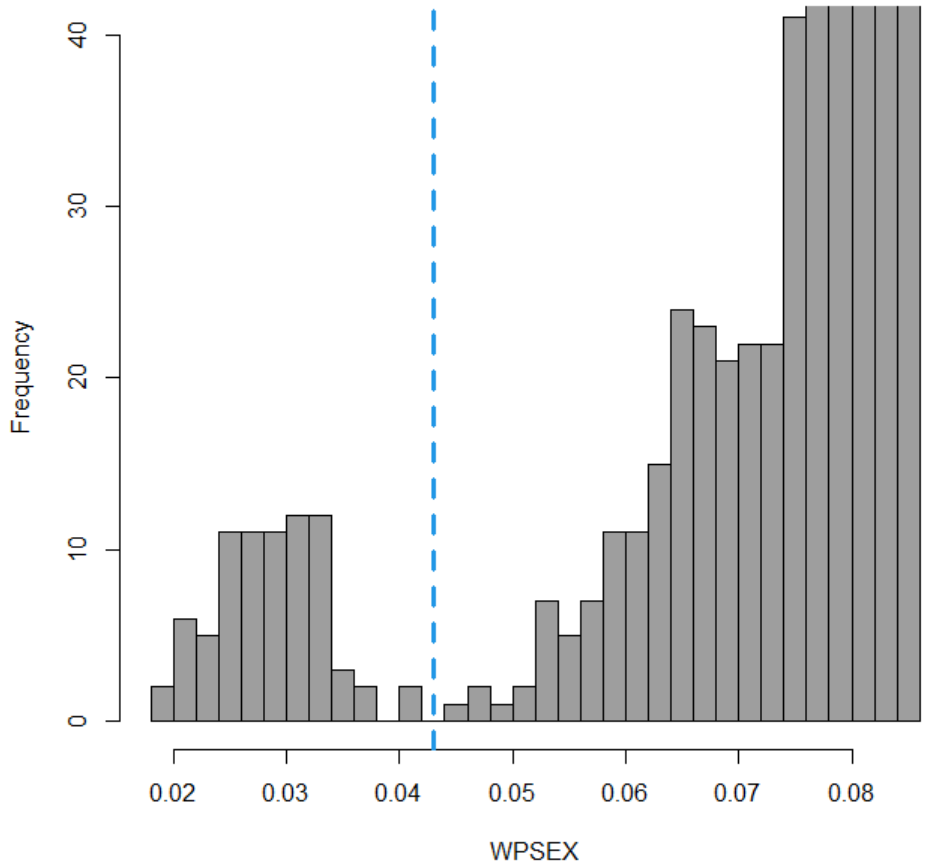

Figure 2. Histogram of the weighted-pseudo-exclusion (WPSEX) statistic for identifying parent-offspring pairs (POPs). Low values (below the vertical blue dashed line) indicate POPs. The x-axis is right-truncated to omit the gigantic peak of unrelated pairs to the right.

Table 3. Number of parent-offspring pairs (POPs) (including those identified using microsatellites and DArTcap data) broken down by juvenile birth year (rows) and adult capture year (columns). NA indicates that no POPs were possible either because no samples exist for that combination of years, or the adult capture year is before the juvenile birth year.

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2016	2017	2018	2019	2020	2021
2002	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2003	0	5	1	2	0	0	0	1	0	1	0	0	0	0	0
2004	0	2	0	0	3	0	0	0	0	0	0	0	0	1	0
2005	1	4	5	4	1	0	0	1	2	0	0	0	0	0	0
2006	NA	4	3	2	0	0	0	0	0	0	0	0	0	0	2
2007	NA	NA	3	4	1	3	2	0	2	0	1	0	0	1	1
2008	NA	NA	NA	NA	0	1	1	1	0	0	0	2	0	0	0
2009	NA	NA	NA	NA	0	1	1	1	0	0	0	0	1	1	0
2010	NA	NA	NA	NA	0	0	1	4	0	2	0	0	1	0	0
2011	NA	NA	NA	NA	NA	0	1	2	1	2	0	0	0	0	0
2012	NA	NA	NA	NA	NA	NA	0	1	1	0	0	1	0	0	1
2013	NA	NA	NA	NA	NA	NA	NA	0	0	1	1	3	1	1	0
2014	NA	NA	NA	NA	NA	NA	NA	NA	0	0	1	0	0	1	0
2015	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0	0	0	0	0
2016	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	2	1	0	1	1
2017	NA	NA	NA	NA	0	0	0	0	0	0	3	0	1	2	0
2018	NA	NA	NA	NA	0	0	0	0	0	0	0	1	2	3	0

HSP-finding

As already noted, there were no additional data on juveniles with which to update the HSP analysis this year.

Summary

This year we successfully completed:

1) The 2023 tissue sampling in Australia (juveniles) but unfortunately only 148 samples were collected in Indonesia in 2022/23 (adults) due to disruptions caused by the Institutional changes in Indonesia.
2) A training workshop for DGCF Enumerators to enable the SBT monitoring and sampling in Benoa to recommence in 2023/24.
3) Tissue subsampling and DNA extraction and sequencing of Indonesian samples collected in 2019/20 and 2020/21 (adults).
4) Kin-finding (POPs only) to include the 2019/20 and 2020/21 adult samples.

An updated dataset of identified SBT POPs was provided to the CCSBT in May 2023, along with the HSPs identified last year (see Farley et al. 2022). To date, a total of 120 POPs and 214 high confidence HSPs have been identified with the false negative rate for HSPs estimated to be 0.25. The results are used in the 2023 stock assessment.

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