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Update on the SBT close-kin tissue sampling, processing and kin-finding 2026

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Abstract

The close-kin project is an ongoing monitoring program that provides data on the adult component of the southern bluefin tuna (SBT) population for use in the Cape Town management procedure and stock assessment models.

Muscle tissue samples collected from harvested juvenile SBT in Australia in 2024 were subsampled, and DNA was extracted and sequenced. The kin-finding analysis to identify parent-offspring pairs (POPs) and half-sibling pairs (HSPs) was updated to include the new juvenile data (n = 1135 new samples after quality control checks). The results were provided to the CCSBT in May 2026. A total of 123 POPs and 230 high-confidence HSPs have now been identified, with a false negative rate for the HSPs of 0.32 (the same as last year). Note the number of POPs has declined by two compared to last year's analysis (Farley et al. 2025a), because no new POPs were identified and two previously-deemed POPs were identified as most likely being full-sibling pairs (FSPs) using more powerful POP-finding statistics.

Between September 2025 and May 2026 (2025/26 spawning season), muscle tissue samples were collected from 5,306 adult SBT in Indonesia. This is well above the usual annual target of 1,500 samples and in line with the proposed number for this season to compensate for low coverage in previous years. It represents a marked increase from previous years and improves coverage for the adult population in close-kin analyses. CSIRO and BRIN have progressed development of a Material Transfer Agreement (MTA) to enable transport of the tissue samples collected since 2022/23 to Hobart for genotyping. We anticipate the genotyping and kin finding will be completed for the 2027 and 2028 CCSBT data exchange.

The 2026 sampling of 1,600 juvenile SBT in Australia has been completed.

Introduction

In 2013, the Extended Scientific Committee (ESC) developed a Scientific Research Plan (SRP) for southern bluefin tuna (SBT), identifying close-kin mark-recapture (CKMR) genetics as a high-priority activity for estimating adult abundance. Since 2014/15, the CCSBT has funded the collection and archiving of SBT muscle tissue, with DNA extraction and sequencing commencing in 2015/16. This work contributed to the completion of a second CKMR abundance estimation project that incorporated both parent-offspring pairs (POPs) and half-sibling pairs (HSPs), 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. In 2019, the CCSBT endorsed an increase in genotyping targets from approximately 2,000 to 3,100 samples per year (combined adults and juveniles) to enhance POP-based cohort comparisons (Anon 2019) and this was formalised as an on-going monitoring activity under the 2023-2027 SRP in 2022.

The monitoring program was disrupted in 2020/21, resulting in reduced tissue sampling (Farley et al., 2022). Following a CCSBT review, a collaborative rebuilding project was initiated to restore

monitoring capacity (Farley et al., 2025b). This paper provides an update on program activities in 2026.

Muscle tissue collection

A total of 5,306 muscle tissue samples were collected from adult SBT at the Benoa Fishing Port in Indonesia between September 2025 and May 2026 (2025/26 spawning season) (Table 1). This is well above the usual annual target of 1,500 samples and in line with the proposed number for this season to compensate for previous years' low coverage (Table 1). Sampled fish ranged in size from 128 to 214 cm fork length (cm).

CSIRO and BRIN have progressed development of a Material Transfer Agreement (MTA) to enable transport of the tissue samples collected since 2022/23 to Hobart for genotyping. We anticipate the genotyping and kin finding will be completed for the 2027 and 2028 CCSBT data exchanges.

The target number of tissue samples from juvenile SBT was met in 2026 with 1,600 samples collected at the tuna processors during harvest operations in Port Lincoln, South Australia. Tissue was obtained from fish ranging from 89-105 cm fork length (FL) to cover the size range of 3-year-olds (see Preece et al. 2024). The tissue samples were collected using the gene-tag tool and frozen according to protocols provided by CSIRO and will be transported to Hobart.

Table 1. Summary of SBT close-kin work undertaken and proposed as part of CCSBT projects since 2015. Note that future genotyping of adult samples is dependent on the MTA being in place in time for sample processing in 2026.

Sampling season	No adults sampled	No juveniles sampled	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	Yes	Yes
2022/23	148	1600	Juveniles completed Adults - late 2026	Juveniles only
2023/24	236	1600	Juveniles completed Adults - late 2026	Juveniles only
2024/25	1148	1600	Planned for 2026	Planned for 2027
2025/26	5306	1600	Planned for 2027	Planned for 2028

DNA extraction and sequencing

Muscle tissue samples from juveniles collected in Port Lincoln (Australia) in 2024 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), of which 1541 are retained for kin-finding analysis. Archive plates of extracted DNA are stored in a dedicated -80°C freezer located at CSIRO Hobart. All sequencing data were sent to CSIRO Hobart in early 2025 for inclusion in the kin-finding (below). There were no new adult data because very few adults were sampled in 2024/25 in Indonesia and the samples are still in Benoa. These samples will be sequenced and included in kin-finding as soon as they are transported to Australia and available for analysis.

Kin-finding

The kin-finding analysis database used for identification of POPs and HSPs was updated to include the DArT sequencing data for juvenile samples collected in 2024.

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 locus, 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 species' allele frequencies as 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, 11,259 adults and 21,611 juveniles remained for kin-finding (Table 2). Note that this table only includes samples that were sequenced using DArTcap and analysed in this report; it does not include samples that were sequenced prior to 2015 using microsatellite markers (recall that the genotyping method changed after 2015 from using microsatellites to DArTcap sequencing; see Bravington et al. 2015; 2017).

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	969	948
2018	700	756
2019	1440	1449
2020	1421	1512
2021	1431	1384
2022	0	1176
2023	0	1143
2024	0	1135
Total	**11259**	**21611**

POP-finding

In previous years, we used a modified Mendelian-exclusion statistic, referred to as the Weighted-PSeudo-EXclusion (WPSEX) statistic (see Appendix B of Bravington et al. 2017), to identify POPs. Because the gap between the WPSEX statistic for POPs and less-related pairs has become less distinct as the number of comparisons has increased exponentially, we used a more powerful Pseudo-Log-ODds (PLOD) statistic this year, similar to the statistic used for finding HSPs except that it measures the relative probability of a pair of fish having their observed genotypes if they are a POP (instead of an HSP) compared to if they are an unrelated pair (UP). This statistic, which we will refer to as $PLOD_{POP:UP}$, was developed after the start of the SBT close-kin program; we did not switch to using it previously since the WPSEX statistic is more intuitive and had been performing sufficiently until recently.

Figure 2 (left) shows the histogram of the $PLOD_{POP:UP}$ statistic calculated for all pairs of adult and juvenile samples that passed QC (21,611 juveniles x 11,259 adults = 243.3 million comparisons). The bump to the right of the dashed blue line (at $PLOD_{POP:UP}$ = 230) corresponds to pairs that are most likely POPs, of which there are 82 pairs. The two pairs with $PLOD_{POP:UP}$ values between 110

and 230 are most likely FSPs. To confirm this, and also check if any FSPs are hidden within the POPs bump, we used another PLOD statistic designed specifically for distinguishing FSPs from POPs, which we will refer to as $PLOD_{FSP:POP}$. We calculated the $PLOD_{FSP:POP}$ statistic for all of the pairs to the right of $PLOD_{POP:UP}$ = 140. The output, shown in Figure 2 (right), indicates there are actually four FSPs – the two already identified plus two others that fell within the range of the POPs bump obtained using the $PLOD_{POP:UP}$ statistic.

Based on these results, the number of POPs identified in the SNP data to date is 80. This is two less compared to last year's analysis (Farley et al. 2025a), because two pairs previously deemed POPs using the WPSEX statistic have now been identified as being full-sibling pairs (FSPs), and no new POPs were found with the data added this year. Including the POPs that were identified at using microsatellites prior to 2015, we now have a total of 123 pairs. The breakdown by juvenile birth year and adult capture year is given in Table 3.

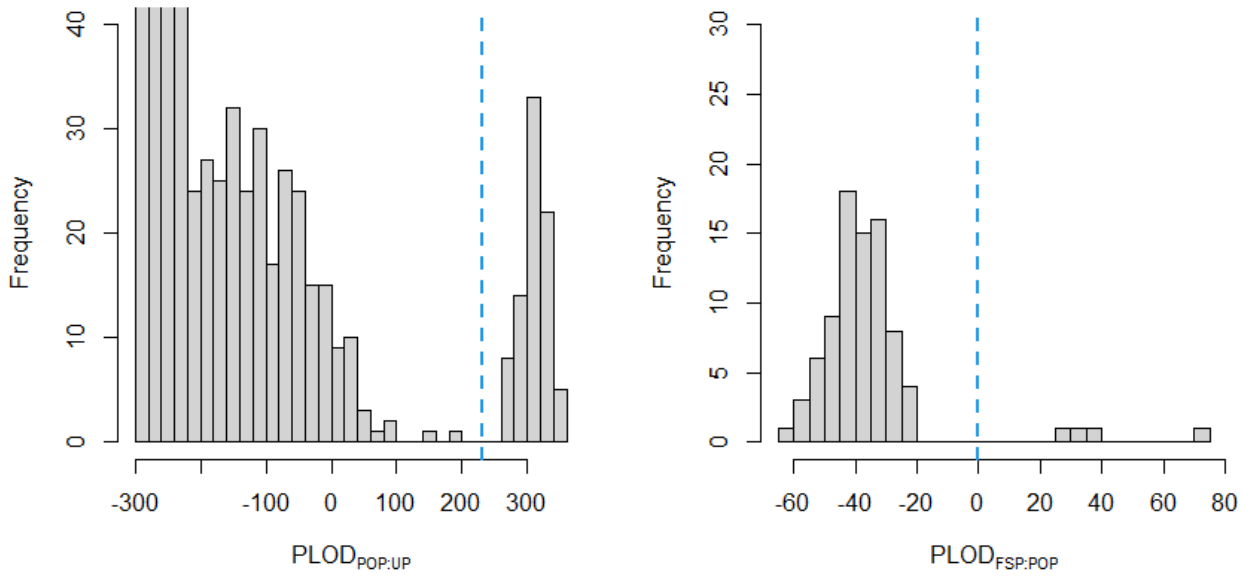


Figure 2. (left) Histogram of the $PLOD_{POP:UP}$ statistic for identifying candidate parent-offspring pairs (POPs). High values (above the vertical dashed blue line) indicate probable POPs. Note the x-axis has been left-truncated to omit the gigantic peak of unrelated pairs (UPs) to the left. (right) Histogram of the $PLOD_{FSP:POP}$ statistic designed for distinguishing full-sibling pairs (FSPs) from POPs, calculated for all potential FSPs and POPs identified in the left histogram (i.e., with $PLOD_{POP:UP}$ values > 140). $PLOD_{FSP:POP}$ values above 0 indicate FSPs and below 0 indicate POPs.

Table 3. Number of parent-offspring pairs (POPs) (including those identified previously using microsatellites – see text) broken down by juvenile birth year (rows) and adult capture year (columns). A blank 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.

	Adult capture year															
Juvenile birth year	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**
2002	0	0	0	0	0											
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	0	0
2005	1	4	5	4	1	0	0	1	2		0	0	0	0	0	0
2006		4	3	2	0	0	0	0	0		0	0	0	0	0	1
2007			3	4	1	3	2	0	2		0	1	0	0	1	1
2008					0	1	1	1	0		0	0	2	0	0	0
2009					0	1	1	1	0		0	0	0	1	1	0
2010					0	0	1	4	0		2	0	0	1	0	0
2011						0	1	2	1		2	0	0	0	0	0
2012							0	1	1		0	0	1	0	0	1
2013								0	0		1	1	3	1	1	0
2014									0		0	1	0	0	1	0
2015											1	0	0	2	0	0
2016											0	2	1	0	1	1
2017												3	0	1	2	0
2018													1	2	3	0
2019														1	0	2
2020															1	1
2021																0

HSP-finding

HSPs were again identified using a PLOD statistic to measure the relative probability of a pair of fish having their observed genotypes if they are an HSP compared to if they are an unrelated pair (UP), which we will refer to as $PLOD_{HSP:UP}$ (see Appendix C of Bravington et al. 2017).

Using the $PLOD_{HSP:UP}$ statistic to compare all possible pairs of juveniles ((21,611 x 21,610)/2= 233.5 million comparisons), we do not get a clear separation between the distribution for HSPs and that for unrelated/less-related pairs, in particular half-thiatic pairs (HTPs, e.g. uncle and half nephew). This has been noted as a problem in the past several years, and the overlap keeps increasing as the number of comparisons increases. Since 2021, we have dealt with this issue by limiting the comparisons to juveniles born less than 9 years apart (or equivalently, sampled less than 9 years apart since all juveniles are age 3 when sampled) (e.g. Farley et al. 2022). Since HTPs are likely to be greater apart in age than 9 years, this reduces the number of comparisons between fish that are potentially HTPs, thus reducing the size of the problematic HTP bump while not excluding too many potential HSPs. To address this issue long term, a high-quality genome assembly for SBT was developed in 2021 in collaboration with the Wellcome Sanger Institute (UK). New algorithms are being developed that leverage linkage information between genetic markers gained from the genome assembly to improve the accuracy of kin-finding. These algorithms have taken longer than anticipated to develop, in part because the approach has morphed over time, and it is uncertain when they will be available for application to SBT.

The $PLOD_{HSP:UP}$ statistic for comparisons between juveniles born less than 9 years apart is shown in Figure 3. The four pairs with values to the right of 150 are full sibling pairs (FSPs)[1]. The division between HSPs and less-related pairs is still not as clear as we would like; however, setting a lower PLOD cut-off value of 55 results in less than 3 expected false positive HSPs and 230 pairs that we are quite confident are HSPs. Note that we used the theoretical means and approximate variances of the PLOD distributions for HSPs and unrelated/less related pairs (calculated as part of the kin-finding software) to determine the expected number of false positives. The breakdown in numbers of identified HSPs by birth year is given in Table 4.

An inevitable consequence of ensuring that false positives are rare is that a reasonable number of false negatives will be present. Using the expected PLOD distribution for HSPs, we estimated the true number of HSPs to be about 32% higher than 230 because of false negatives. Although having such a high false-negative value is not ideal, it can be accounted for within the population modelling (Bravington et al. 2017).

[1] Note that all four FSPs were within-cohort pairs, as one would expect for a large adult stock.

Table 4. Number of half-sibling pairs (HSPs) broken down by birth year of younger sibling (rows) and older sibling (columns). Note that comparisons were only made between juveniles born less than 9 years apart.

Birth year	Birth year older sibling																		
younger sibling	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
2003	2	4	2	1	0	0	1	0	0										
2004		5	2	6	2	2	1	0	0	2									
2005			5	3	3	3	0	4	1	0	0								
2006				8	3	1	3	4	3	0	1	1							
2007					3	3	2	1	2	2	2	1	2						
2008						5	1	0	2	3	0	1	0	2					
2009							1	1	1	0	0	0	0	4	2				
2010								2	1	2	1	0	1	1	0	0			
2011									3	2	1	0	2	4	1	0	0		
2012										2	2	1	1	1	2	1	0	0	
2013											2	4	0	0	1	0	0	0	0
2014												2	2	1	4	3	1	0	1
2015													4	2	1	3	0	0	2
2016														6	3	4	6	1	0
2017															5	2	3	4	1
2018																5	2	0	3
2019																	4	1	0
2020																		0	2

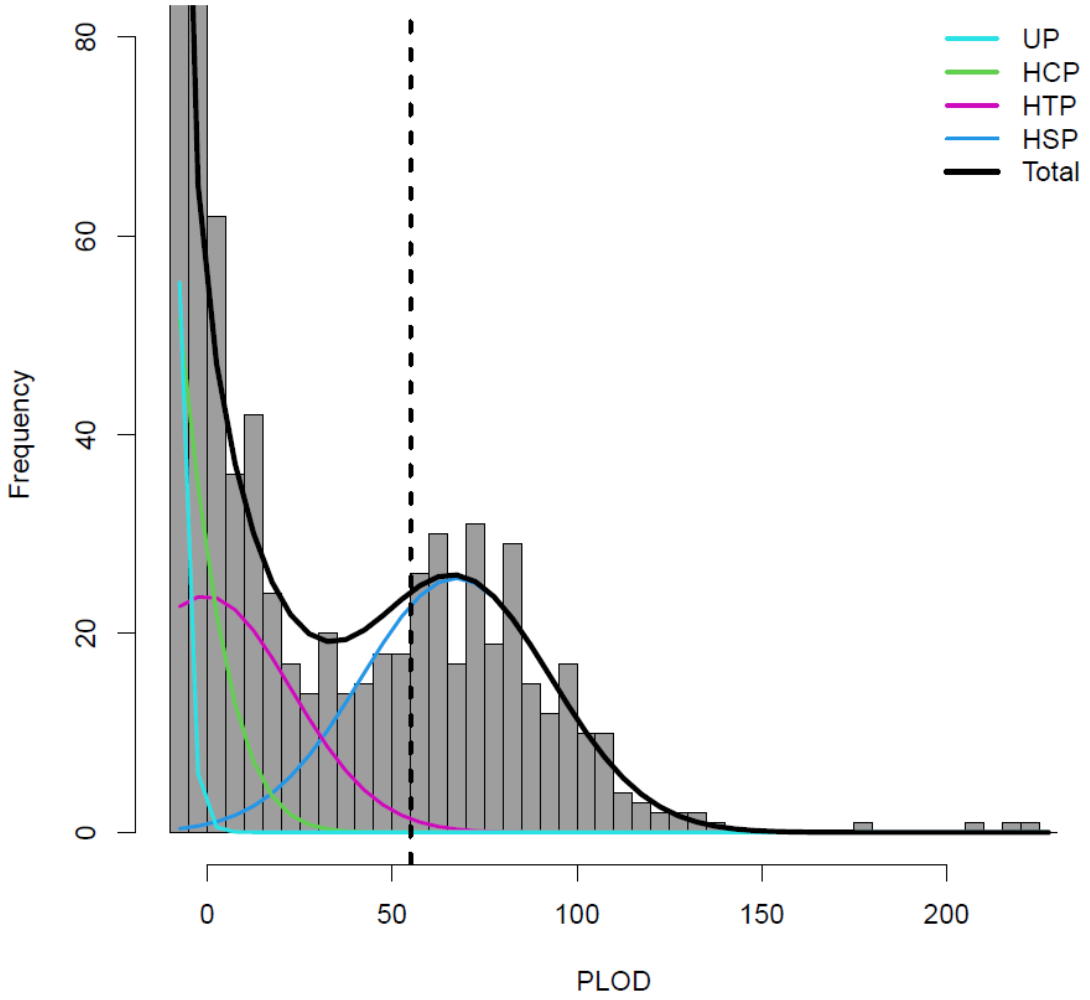


Figure 3. Histogram of the $PLOD_{HSP:UP}$ statistic for pairwise comparisons of juveniles born less than 9 years apart. The approximate PLOD distributions for unrelated (UP), half-cousin (HCP), half-thiatic (HTP) and half-sibling (HSP) pairs are shown. With a lower $PLOD_{HSP:UP}$ cut-off value of 55 for HSPs (vertical dashed black line), we expect <3 false-positive HTPs. The four pairs with $PLOD_{HSP:UP}$ values > 150 are full-sibling pairs (FSPs). Note that the x-axis is left-truncated to omit the gigantic peak of UPs to the left.

Summary

This year we successfully completed:

1) The 2026 tissue sampling in Australia of 1,600 juveniles.
2) The 2025/26 tissue sampling in Indonesia of 5,306 adults.
3) Tissue subsampling and DNA extraction and sequencing of juvenile samples collected in Port Lincoln in 2024.
4) Kin-finding (POPs and HSPs) to include the 2024 juvenile samples.

An updated dataset of identified SBT POPs and HSPs was provided to the CCSBT in May 2026. To date, a total of 123 POPs and 230 high-confidence HSPs have been identified, with the false negative rate for HSPs estimated to be 0.32.

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