PACIFIC TUNA TAGGING PROJECT

WP 6 CRUISE REPORT

14 September- 07 October 2022

INTRODUCTION

This report summarizes activities of the Pacific Tuna Tagging Project (PTTP) Phase 2- **WP6 cruise** during a 24-day period, following a departure from Noro on the 14th September, fishing in Solomon Islands waters. The vessel returned to Noro on the evening of October 7th.

Scientific SPC and in-country personnel onboard the Soltai 105 during the cruise are listed in **Table 1.**

Table 1. SPC and In-Country personal onboard during Cruise 1

Name	Title	Affiliation
Bruno Leroy	Cruise Leader	SPC
Aurelie Guillou	Senior Fisheries Technician	SPC
Joe Scutt Phillip	Senior Fisheries Scientist	SPC
Marion Boutigny	Tagging Technician	SPC
Jeff Muir	Tagging Technician	SPC contractor
Lindon Havimata	Fisheries Researcher	SINU
Patteson Omi	Scientific Observer	MFMR

GENERAL DESCRIPTION OF SEARCHING AND FISHING ACTIVITY

The vessel departed Noro on mid-afternoon of September 14th, directly steaming to the baitground in Munda.

Utilizing Patutiva baitground, four days were spent fishing with good success in the south of New Georgia, Rendova, Tetepare and Vangunu islands. One half-day on the 19th was lost in Noro to replace an essential electric component of the cooling system after the brine temperature got too high and an alarming amount of ammonia was detectable (by smell) on the vesselBecause of this uncertainty with the refrigeration system, the team decided to offload biological samples in Noro in a more stable freezer situation.

Following this, four days of very poor fishing were experienced around Vella Lavella, the southeast of Choiseul and north-west of Isabel before returning to the south of Vangunu and the productive free schools previously fished there. The vessel then fished the southeast point of Isabel and a seamount between Isabel and Malaita from the San Jorge baitground before switching to Taroniara baitground and Indispensable Strait between Malaita and Guadalcanal. Many cooperative free schools were fished and tagged in this area, despite more time lost with fuel delivery issues in the main engine resulting in loss of hull speed. The final four days were spent fishing north Russell and Vangunu islands, noting that 2 additional half days (mornings) were lost on the 2nd and 6th of October due to main engine problems with multiple shorter incidents on other days.

The track of the cruise, based on schools and 1800 positions, is found below (in **Figure 1)** and a summary of general movements and tag releases follows in **Table 2**.

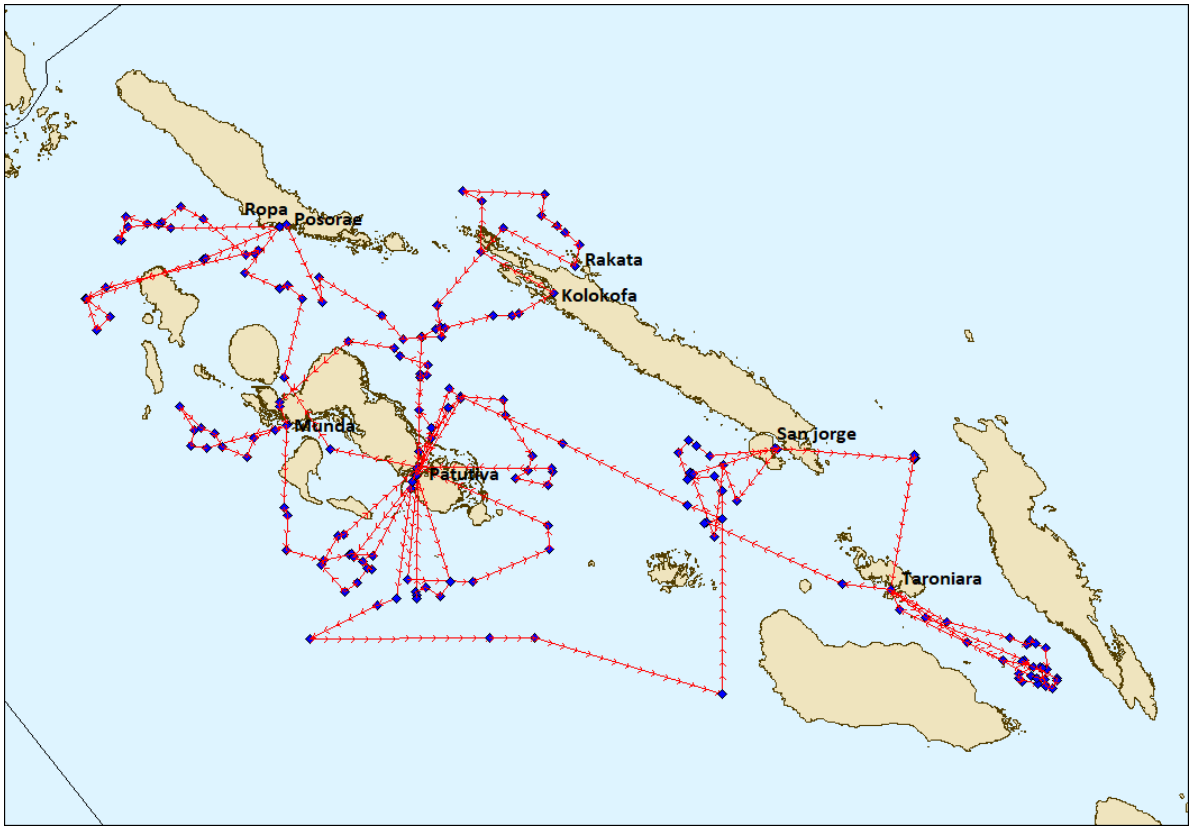


Figure 1: Cruise track (2558 nautical miles) during WP6 with events and baitground positions

Picture 1: FV S105 in action

Table 2: Summary WP6 activity, with the number of conventional tag releases per day

Date	Area	Baitgrounds	Notes	Tags
14-Sep	leave Noro 3pm	Munda		**0**
15-Sep	WSW Munda- Simbo Fads	Munda		**640**
16-Sep	South Rendova, Tetepare	Patutiva		**43**
17-Sep	South Tetepare/Vangunu	Patutiva		**2113**
18-Sep	South Vangunu	Patutiva	After baiting steam towards Ropa	**326**
19-Sep	Noro (am), Slot	Ropa	Half-day in port for engine repair	**0**
20-Sep	North Vella Fads	Ropa		**24**
21-Sep	West Vella Fads & seamount	Posorae	After baiting steam towards Kolokofa	**64**
22-Sep	Slot between Posorae & Kolokofa	Kolokofa	After baiting steam towards Kologilo Pass	**1**
23-Sep	Fish towards Rakata	Rakata	After baiting steam towards Kologilo Pass	**4**
24-Sep	Fish towards Seghe	Patutiva		**39**
25-Sep	Fish NE Vangunu	Patutiva		**191**
26-Sep	Fish South Tetepare	Patutiva		**1640**
27-Sep	Fish SE Vangunu		Steam towards San Jorge overnite	**254**
28-Sep	SE Isabel	San Jorge		**754**
29-Sep	SE Isabel	San Jorge		**1305**
30-Sep	Fish between San Jorge & Malaita	Taroniara		**54**
1-Oct	Fish along west Malaita	Taroniara		**2423**
2-Oct	Fish Indispensable Strait	Taroniara		**1775**
3-Oct	Fish Indispensable Strait	Taroniara		**1688**
4-Oct	Fish North Russell Fads		Steam towards Marovo overnite	**1718**
5-Oct	Fish north Vangunu	Seghe		**287**
6-Oct	Fish north Vangunu	Seghe		**292**
7-Oct	Fish toward Noro		Arrival in port at 21:30	**1342**
Total Tags				**16977**

TAG RELEASES

Of the 24 days of the cruise, 23 days were spent searching and fishing including one half-day in port and two other half-days waiting for engine repairs. A total of 16,977 fish were tagged and released during the cruise, at an average of 738 fish per day, across 67 separate events. The species composition in total was 98% skipjack and 2% yellowfin with only 2 bigeye tuna tagged. Only 1.5% of the releases used the smaller 11 cm tags (fish size < 38 cm FL). 21% of releases were in association with anchored FADs (22 events), 1% with seamount, (2 events) and the free school releases represented the large majority of 78**%** (43 events)**.** The releases are summarized by school location, school type, day and species in **Appendix 1**, whereas **Figure 2** below shows the spatial distribution of releases during the cruise.

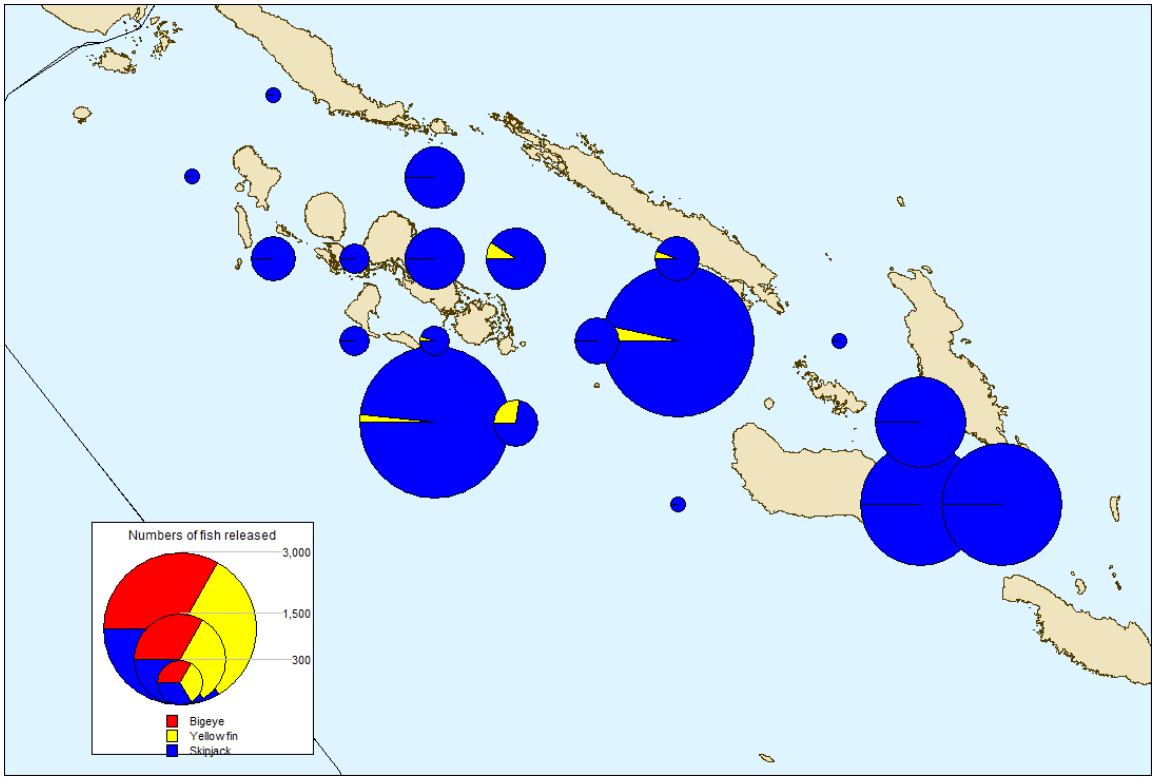
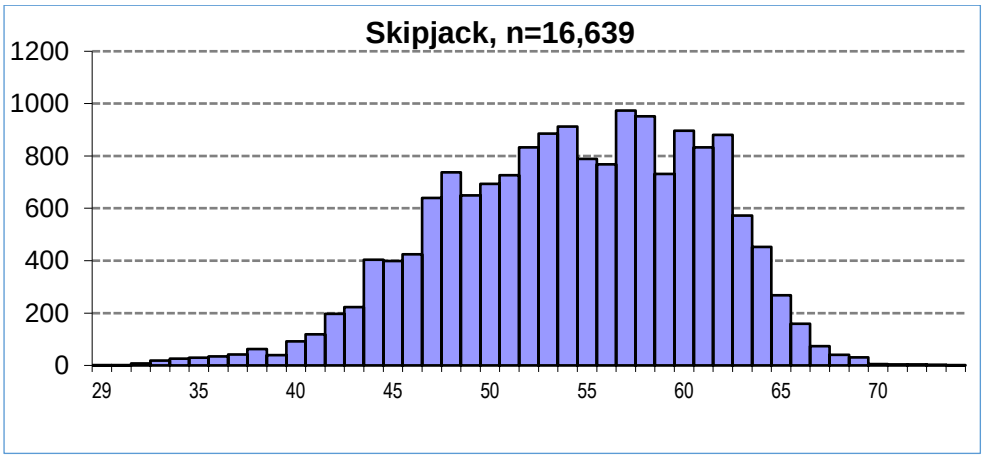


Figure 2: Spatial distribution of tag releases by species during WP6, by 1° grid cell

SIZE DISTRIBUTION OF TAGGED FISH

Figure 3 shows the size distribution of fish tagged during WP6. The skipjack size distribution was multi-modal whereas the yellowfin sizes were mostly small fish (35-45 cm) with very few fish over 50 cm.



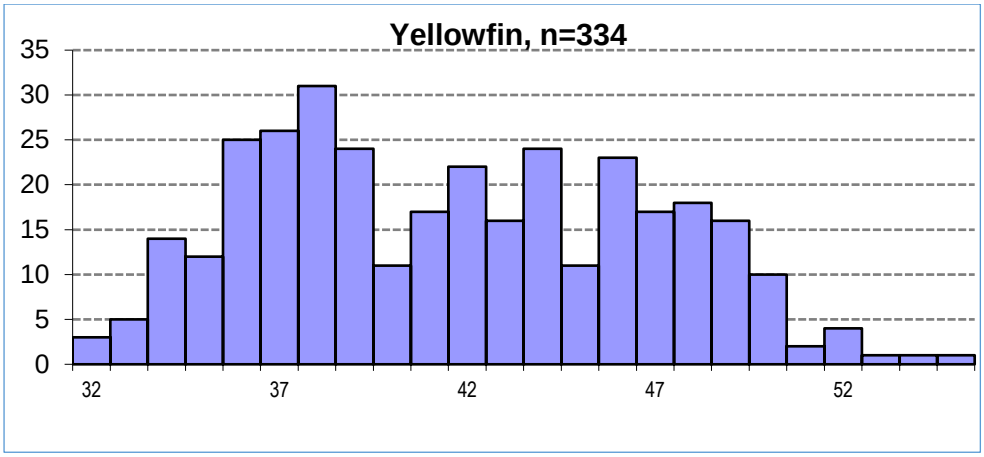


Figure 3: Size distribution of tagged fish during WP6

BIOLOGICAL SAMPLING

1. Routine sampling (for the WCPFC Pacific Marine Specimen Bank)

Routine sampling is a standard protocol performed on all PTTP tagging cruises. As soon as possible following a tagging event, the science team assesses the number of fish caught during the event and, if possible, starts the routine sampling on target species (SKJ, YFT and BET) and any bycatch available. This protocol collects the normal suite of samples from 10 specimens per species, and for WP6 this including a genetic sample taken with a single-use biopsy punch. All samples except otoliths were immediately frozen. The standard operating procedure (SOP) for routine sampling can be found in the biological sampling plan, **Appendix 2** of this report.
In total, 257 fish were sampled, including 230 SKJ and 24 YFT (see **Table 3** about type and number of collected samples). Only 3 bullet tuna were sampled as bycatch species, with no other bycatch available during fishing. Biological sampling was conducted on 28 of the 67 total tagged schools during the cruise.

	No. of samples collected						
Species	**Gonad**	**Stomach**	**Livers**	**Dorsal spines**	**Otoliths**	**Muscles**	**Biopsies**
BLT	0	3	2	0	0	3	0
SKJ	229	230	230	230	221	230	230
YFT	24	24	24	24	20	23	24
Grand Total	**253**	**257**	**256**	**254**	**241**	**256**	**254**

Table 3: Total number of samples per type and per fish

Genetic sample additions to bio-sampling

In addition to standard bio-sampling practices, each specimen had an additional tissue sample taken explicitly for genetic analysis. The SOP was interpreted from the biological sampling plan (appendix 2) by each sampler (see below, *inter-sampler variability*). Tissue samples were taken with single-use, 3mm biopsy punches and then stored in a 2mL cryovial containing RNA-later preservative. After sampling, the vials with samples were stored in a freezer.

2. Inter-sampler variability in DNA contamination

Each of the seven scientific staff on board were asked to read and interpret individually the SOP for taking samples for genetics using single-use biopsy-punches, and collect at least 30 samples per person. Analysis will be performed to assess the level of inter-sampler variability that would preclude biopsy sampling onboard commercial fishing vessels by ordinary observers or fishermen, without contaminating genetic material. By engaging the science team in the biopsy punch sampling during WP6, the experiment will allow a comparison of sample cross-contamination rates when different people independently follow the same genetics sampling protocol.

Sampler	Genetic samples collected
AUG	33
JFM	44
BML	22
JSP	31
LNH	34
MAB	51
PAT	42
Grand Total	**257**

Table 4: Number of genetic Samples Collected by Science Team by Member

3. Fatmeter measurements

WP6 also provided an opportunity to augment the numbers of Fatmeter measurements for tunas captured in the Solomon Islands EEZ. Data on tuna fat content provides insight into the quality and quantity of prey available in the environment and on the body condition of tunas, and so represents a potentially valuable ecosystem indicator.

The objective during WP6 was to obtain accurate Fatmeter measurements on as many SKJ, YFT and BET as possible as they came aboard during a fishing event. Measurements were taken from fish in two different ways. First, fish selected for routine sampling were also measured for fat content. In addition, and separately, a second group of fish was targeted for fatmeter and length measurements only, when excess were available on deck immediately following a fishing event, or when time between events did not permit routine sampling. 232 fish were measured in the former group (211 SKJ, 21 YFT), and 428 fish in the latter (424 SKJ, 4 YFT). The length frequency of fatmeter measured fish is shown in figure 4.

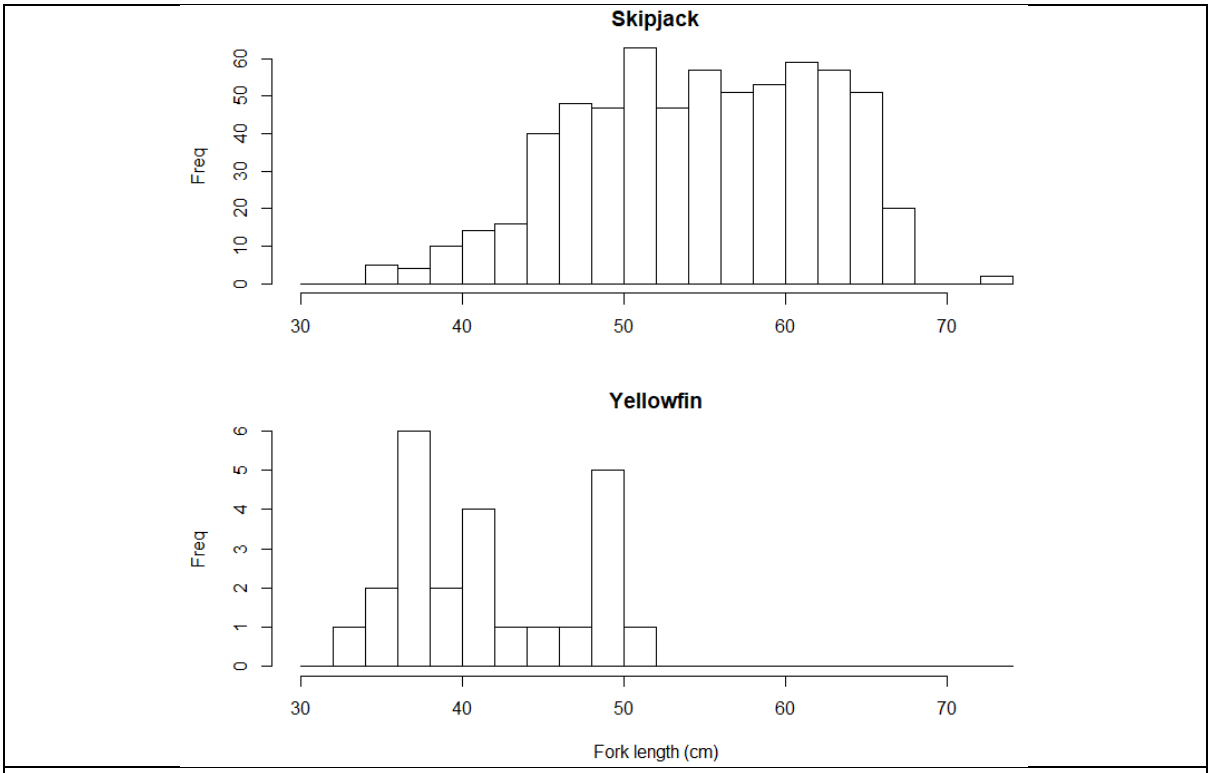


Figure 4. Length frequency of fish with fatmeter measurements during WP6

4. $SrCl_2$ marking and biopsy sampling at release

Good numbers of recaptures from $SrCl_2$ marked SKJ from the Solomons' EEZ will contribute important data to the ongoing SKJ otolith ageing work. Growth between the day a fish was tagged and the day it was recaptured are inferred from the position of the $SrCl_2$ mark on the otolith. Additionally, taking a genetic sample from these $SrCl_2$ marked fish at release, and using the tag recovery network to ensure a muscle sample can be collected at recovery, will provide much-needed samples for validation of the epigenetic ageing model for SKJ.

During the cruise, 630 tagged skipjack were injected with strontium chloride ($SrCl_2$) prior to release with white 13 cm conventional tags, of which 312 were also sampled with a live biopsy. $SrCl_2$ marks the otolith of the fish the moment it is tagged. Once the fish is recaptured by a fishing vessel, an attempt is made at recovering the intact carcass, and the otoliths can be extracted from the specimen. **Table 5** provides the number of $SrCl_2$ marked skipjack tuna, separated by school type.

Skipjack of medium size (between 37 cm and 64 cm) were targeted for $SrCl_2$ injections and live biopsy. Fish were injected with $SrCl_2$ dorsally of the lateral line using a veterinarian-style manual syringe gun, fed from a reservoir with plastic tubing. The volume of each injection depended on the size of the fish (see **appendix 2**), using up to 2 injections of 6 ml for fish of 64cm.
Genetic sampling via live biopsy was performed by a second technician at the cradle, building on a protocol developed during a previous central Pacific cruise (CP14). During WP6, biopsies were taken using a CSIRO widget tool with disposable and sterile plastic tips that are released from the widget once the live biopsy sample was taken. Initially, 281 live biopsies were taken with the widget and, after running out of tips, 31 additional live biopsy were taken using standard biopsy punches. Tips and biopsies were stored in 2ml cryotubes filled with RNA-later, and refrigerated.

	Free schools	FAD	Seamount	Total
$SrCl_2$ **Tagged SKJ**	475	151	4	630

Table 5. Number of $SrCl_2$SKJ released per type of school association

A great effort was made to increase the efficiency and number of $SrCl_2$ marked fish with live biopsy sampling during WP6. While the ambitious target of 2000 releases was not met, the rate of releases was increased from 3% to 4% of total skipjack releases, compared to previous experiments during WP5. A live biopsy was taken from almost 50% of these fish, as this procedure is slower than injection and tagging. In addition, it was noted that biopsy punches were not as efficient or fast as the widget tool, increasing the chance of cross contamination and failure to collect samples in adequate time. This also increased the time of releasing the fish and may therefore influence the individual's chances of survival.
A variety of factors influenced the ability to perform $SrCl_2$ injections and live biopsy samples; 1) the condition of the fish landed on cradle has to be adequate to carry out the procedure, 2) the individual must be of appropriate size, 3) there must be enough time between successive fish landed on the cradle to undertake the procedures, and 4) fish must be in a reasonably calm state.
During pole and line operations, controlling these factors is difficult. In particular, the speed and irregular flow of the fishing and arrival of fish at the cradle make maximising the release of $SrCl_2$ marked fish with biopsy samples challenging. It is critical for the tagger in charge of the $SrCl_2$ injections to switch between normal conventional tagging and $SrCl_2$ injections in order to control this flow and select the best fish suitable for the procedure.

While some small improvements may still be possible to slightly increase the proportion of released fish with $SrCl_2$ injections and live biopsy samples, under current operations it appears that the maximum number possible may be near it's limit. The use of the widget and tips for live biopsies is recommend for its speed and ease for the sampler. The development of smaller tips should be explored to ensure less damage to the tagged fish, alongside alternatives to using soap to clean the widget to reduce slippage.

If greater proportion of $SrCl_2$ marked fish are needed for future experiments, implementing an extra, dedicated cradle at midships with its own assigned fishers and handlers should be considered. This was suggested by the crew as a potential way to ensure a more constant and manageable flow of fish for this procedure.

5. SKJ stomach microbiome experiment

Canberra University asked SPC to lead an experiment on the stomach microbiome of skipjack tuna. The aim was to explore the effects of time post-capture and storage on the stomach microbiome of this species, collected under normal fishing operations. Two different sample collections were completed by the WP6 team. Post processing of samples will be conducted by Canberra University.

Experiment 1

Experiment 1 aimed to collect the stomach juices of 50 skipjack tuna from a single school. All 50 individuals had to be of a similar size in order to control the variation in size-related diet preferences. The SKJ microbiome was then sampled in five sampling events, as follows:

- 10 fish sampled at time 0 min after catching. The team processed all of them and put the microbiome tubes in RNA later and stored it in the freezer
- 10 fish sampled at time 2h after catching. The fish were put in a tray for 2 hours in the shade and covered with a wet towel. After that, the team processed the fish.
- 10 fish sampled at time 24 hours after catching. After being caught, the 10 fish were frozen into the brine for 24 hours and defrosted for 2 hours on the deck. After that, the team processed the fish.
- 10 fish sampled at time 12 days after catching. These 10 fish were intended to be processed on September 29, so 12 days after the catch. On September 19th, the boat had a problem with the cooling system and the well's temperature reached +5°C. Facing this problem, the team decided to process these 10 fish on September 19, so only 3 days after the catch.
- 10 fish sampled at time 24 days after catching. The team decided to keep these fish frozen at port and processed the fish on day 22 after the end of the cruise.

Fish were sampled to emulate as closely as possible typical fishing operations and sampling of whole stomachs by onboard or port samplers. First, fork lengths were taken for each individual, and then an incision was made using a knife to open the ventral cavity to expose the organs. A second incision was then made posterior to the pelvic fins towards the head to expose the heart. A second technician, wearing a clean pair of surgical gloves, then removed a roughly one centimetre piece of heart using sterile forceps and scissors. Following this, the stomach was then carefully removed using the same knife, and placed on a clean layer of aluminium foil. The stomach was then cut open by the second technician using the same forceps and scissors, and after removing any large prey items, the stomach lining was scraped using a sterile plastic spoon and emptied into tubes containing RNA-later. Forceps, scissors, surgical gloves and the aluminium foil were changed between each individual.

Experiment 2

Experiment 2 aimed to sample 10 fish from the same school, collect the stomach microbiome, and mix it with MilliQ water before freezing it. At 5 different times during the cruise (0 min, 2 hours, 24 hours, 12 days and 24 days), the team defrosted 10 vials, one from each fish, and pour the content of each vial into vials with RNA later. All RNA later tubes were returned to the freezer afterwards.
This experiment wasn't impacted by the breakdown of the onboard cooling system, because no frozen fish were used for this exp. 2.

<u>Issues with WP6 biological experiments and sampling</u>

1. For the next cruise, it would be more efficient to have pre-filled vials of RNA-later to fill up the vials, saving time during the cruise schedule.
2. On WP6 cruise, 6 litres of SrCl solution were available for 1 month cruise and this quantity was more than enough.

3. The team ran out of widget tips, which were far superior to the use of biopsy punch tools for live biopsy taking. In the future, the appropriate amount for the number of samples required should be loaded on the vessel.

BAITING

All "active" bait grounds must have a signed agreement between their owner(s) and NFD before one of the P&L boats can come to baitfish for bait in the area; this agreement usually includes a fixed baiting night fee. On the eight different visited bait grounds (see **Figure 1 and 5**), most provided excellent catch amounts of good bait species and size (see **Table 6**). A special mention must go to Taroniara, which delivered very nice blue anchovies into the net for three nights. This bait species certainly contributed to the fishing success that was obtained on numerous encounters with free schools in the Indispensable Strait even, despite boat speed being limited to 6 knots during most of that period due to problems with the main engine.

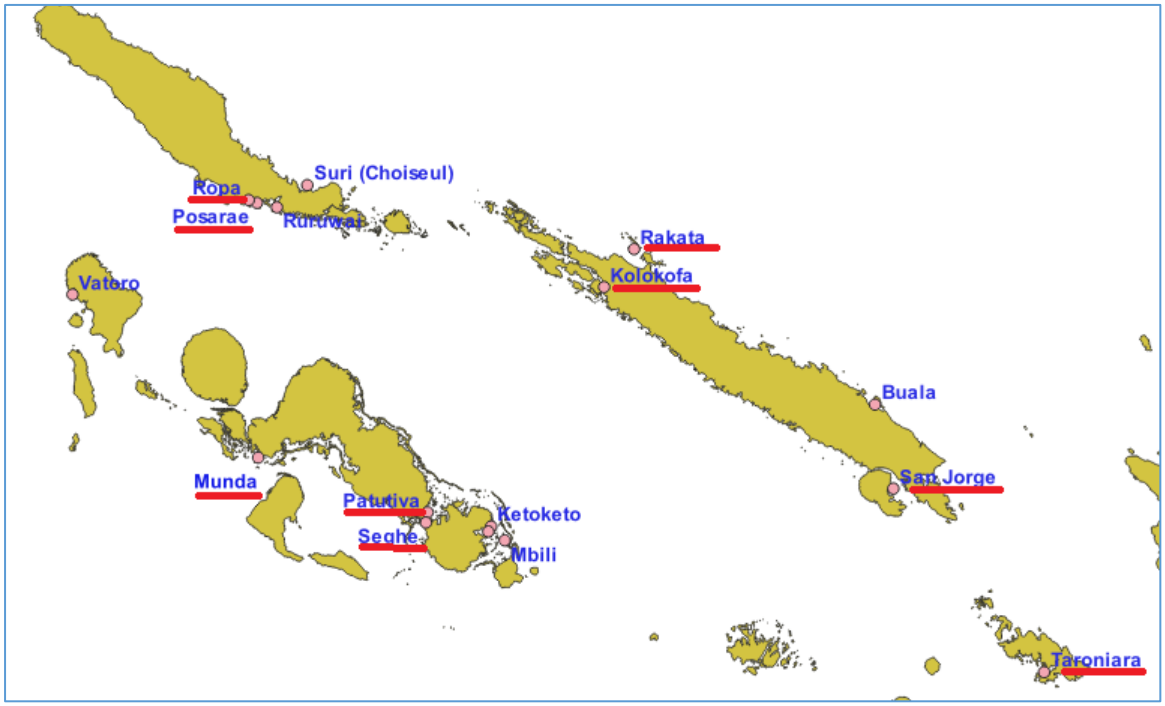


Figure 5: Visited bait grounds during WP6 cruise (underlined in red)

Table 6: Summary of bait fishing activity during WP6 cruise

Date	Location	Catch (Bkts loaded, hauls)	Species
14-Sep	Munda	250 (1)	Gold anchovies (devisi)
15-Sep	Munda	160 (2)	anchovies (devisi)
16-Sep	Patutiva	191 (1)	anchovies (devisi)
17-Sep	Patutiva	274 (2)	anchovies (devisi)
18-Sep	Patutiva	21 (1)	anchovies (devisi)- Poor catch due to bait net tangled
19-Sep	Ropa	160 (1)	devisi, sprats (lewisi), fusiliers, Pellona, rastrelligers
20-Sep	Ropa	180 (2)	devisi, fusiliers, pellona
21-Sep	Posorae	71 (2)	devisi, hardy heads, blue sprats
22-Sep	Kolokofa	126 (2)	devisi, hardy heads, blue sprats
23-Sep	Rakata	70 (2)	small blue anchovies, sprats (lewisi) and hardy-heads
24-Sep	Patutiva	170 (3)	anchovies (devisi)
25-Sep	Patutiva	217 (3)	anchovies (devisi)
26-Sep	Patutiva	249 (2)	anchovies (devisi)
28-Sep	San Jorge	290 (2)	gold anchovies, rainbow sardines, pellona, blue sprats
29-Sep	San Jorge	212 (2)	Devisi, rainbow sardines, pellona
30-Sep	Taroniara	247 (2)	anchovies (heterolobus, few devisi), S gracilis, few hh
1-Oct	Taroniara	305 (2)	anchovies (heterolobus, few devisi), S gracilis, few hh
3-Oct	Taroniara	256 (2)	anchovies (heterolobus, few devisi), S gracilis, few hh
5-Oct	Seghe	166 (2)	devisi, blue sprats
6-Oct	Seghe	300 (2)	devisi, blue sprats, few small rastrelligers.
Total		**3915 (38)**	

CONCLUSIONS

WP6 was a busy research cruise, with no noticeable down-time, aside from those caused by mechanical issues. It combined efficient fishing/tagging operations and an ambitious biological sampling and science program. Although comprising seven science staff (the biggest science team in PTTP history), and assisted by many crew members familiar with the experiments, the research team approached the limits of what can be accomplished onboard such a commercial fishing vessel.

The exceptional persistent good weather conditions (most of days with less of 10 knots winds) and the abundant quantity of encountered skipjack free schools (except in the north of Isabel Island) permitted the completion of the research objectives, both in term of tagged fish numbers and in term of biological experiments.

The low response and hook-up rate from the numerous schools encountered around Vella Lavella Island may be explained by the presence of a considerable percentage of larger yellowfin tuna in the area, which are well-known to keep schools just outside of the boat sprayers. This led to very frustrating days during which tags could not be deployed in this area where there were obviously large quantities of fish.

The most remarkable fact, and with no obvious explanation, was the absence of tuna in the north side of Isabel Island during the days spent searching there. Our observations were confirmed by the simultaneous presence in the area of the FV Solomon Fisher, used by NFD for FAD maintenance and tuna school presence information, both on FADs and in free schools. According to the Soltai 105 Fishing Master Grey M, the NFD purse seine boats have stopped fishing in this area during the last 3 years…. There may be oceanographic or other tuna habitat information that can be examined in this region to shed light on such an apparent shift on an area that has previously supported many tag releases.

The NFD engineers had clearly worked very hard to get the Soltai 105 as reliable as possible for WP6, but it is obvious that the boat is now facing serious difficulties due to the lack of funds budgeted for insuring the maintenance of the last pole and line fleet in the region. The 2 sister ships S101 and S105 are now 16 years old but are also unlikely to last very much longer without some serious investment. These vessels are not currently in a condition that will allow any oversea charters to be envisaged for PTTP. However, the proximity to Noro and the number of NFD vessels in the Solomons EEZ demonstrated that even this aging vessel (see **Picture 1**) with many mechanical issues was quite able to complete the objectives of the trip, a convenient situation that should not be overlooked for future work where overseas travel is not necessary.

Nevertheless, the enthusiasm and competency of the S105 crew was once again at the forefront of the success of WP6. Thanks must go to the NFD management and engineering team for their hard work during boat pre-cruise preparation. Comparing the operational state of the unfortunate Soltai 101 helps understand the amount of work that was involved in preparing the S105!

Some luck was had due to the high time that NFD vessels spent in port during the cruise dates, although several large catches of tagged fish were still made. In particular, the presence of fish in the south Vangunu island and the Slot areas appears to have driven NFD purse seine vessels to fish and recapture WP6 tags released there (see **Figure 6**). As of the 18th October 2022 (10 days after the completion of the cruise), approximately 1800 tags have been reported as recaptured, representing 11% of the total releases. This is larger than for the same period post WP4 (< 1% recaptures 10 days after completion), although this did rise to 4.5% after two months.

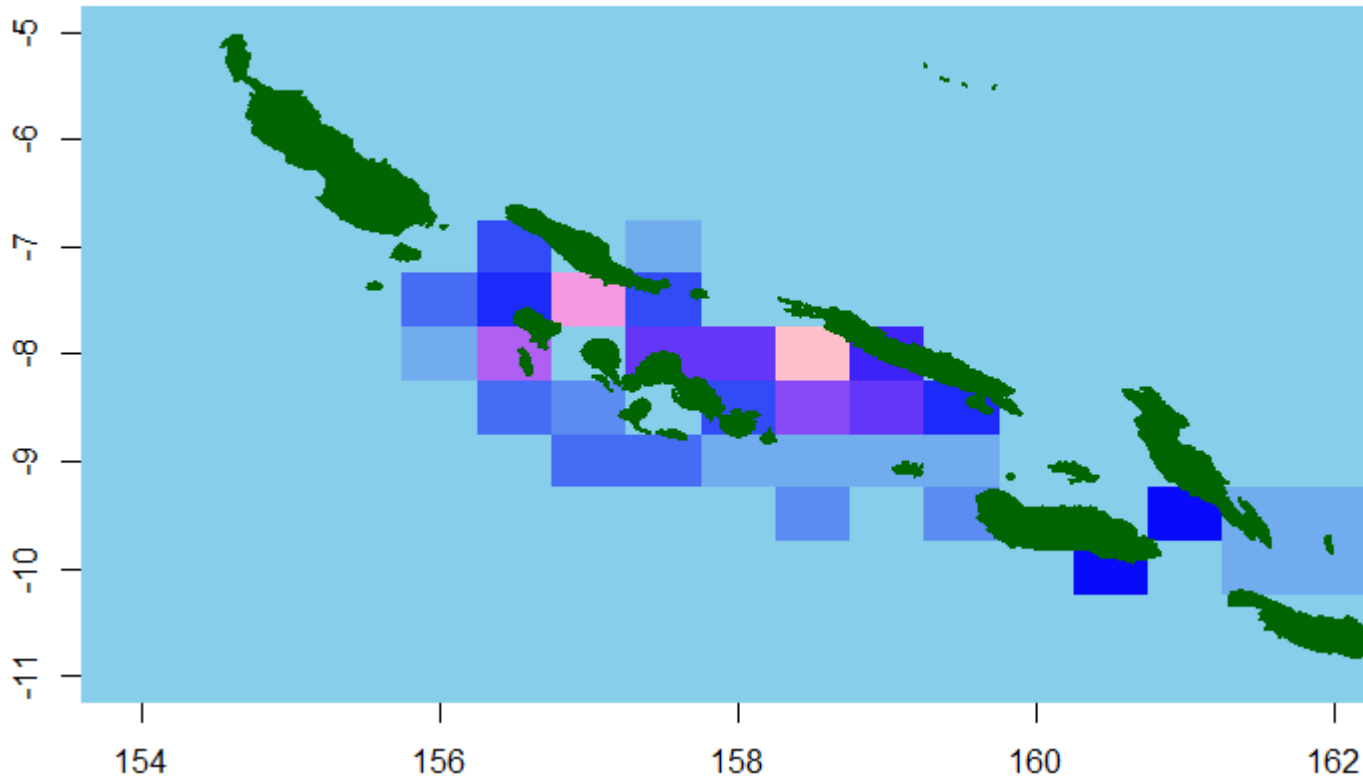


Figure 6: Estimated days fishing activity by NFD purse seine vessels during WP6, aggregated to ¼° cell

<u>List of boat engine failures (necessity of urgent repair) during WP6:</u>

1. Electrical problem in the cooling system leading to pressure irregularity and leak of ammonia. This required the return to Noro to obtain spare parts (**Loss of the 19 Sep morning**), and in one instance the momentary clearing of the midships deck due to the perceived dangerous levels of ammonia.

2. Fuel pump bleed-valve stripped threads. The boat had to stop in emergency to repair

3. High exhaust gas temp (EGT) caused by excessive fuel pressure and the need to replace the tip of one injector (**Loss of the 2 Oct morning**)

4. Fuel pump adjustment of rack and tired injectors (should have been replaced) and turbo problem. Had to reduce speed from the 2nd of Oct to 7 knots then to 6 knots from the 3rd of Oct

5. Airlock in one cylinder (**Loss of the 6 Oct morning**)

6. Injectors rebuilt with spares and old parts multiple times

7. Low pressure oil line from oil filter gasket leak

Other noticeable problems:

1. They were no operational radio communications: the long-range unit had no reception/emission and VHF had only a couple of nm range. This is of course of safety concern, but it was also frustrating during WP6 operations as it was impossible to communicate with other boats from the fleet to get regular fishing updates. All communications had to go through email system or from personal mobile phones at the baitgrounds.

2. -The Bouke ami boom has broken twice (21 and 22 Sep): the original material is now very fragile (brittle) after all these years under the sun. Clearly needs to be replaced.

3. Weak fish well refrigeration system (not below -14 C), although not a real concern for PTTP research cruises at present.

4. The engine control at the fly bridge still not replaced, although rusted to death (since 2017)

5. Many fittings, plates, davits etc are rotted and corroding badly and should be assessed for safety of the crew.

Appendix 1

Releases of tagged fish by date, event start time, location, school type and species

Date	start_time	event_lat	event_lon	Sch_Ass	SKJ	YFT	BET	TOTAL
15-Sep-22	0635	0826.463S	15645.224E	Anchored FAD	**49**			**49**
	1115	0821.300S	15648.400E	Free school	**12**			**12**
	1330	0826.900S	15654.700E	Anchored FAD	**454**			**454**
	1637	0824.280S	15704.200E	Free school	**125**			**125**
16-Sep-22	0624	0847.000S	15714.160E	Anchored FAD	**42**			**42**
	0755	0857.380S	15713.800E	Anchored FAD	**1**			**1**
17-Sep-22	0610	0906.730S	15802.860E	Anchored FAD	**9**	**27**		**36**
	0731	0911.075S	15759.770E	Free school	**457**			**457**
	0853	0908.290S	15755.420E	Free school	**401**	**9**		**410**
	0954	0909.700S	15752.490E	Free school	**838**			**838**
	1215	0911.540S	15752.780E	Free school	**372**			**372**
18-Sep-22	1614	0857.070S	15832.300E	Free school	**326**			**326**
20-Sep-22	0949	0721.900S	15626.400E	Free school	**2**			**2**
	1222	0720.332S	15636.423E	Free school	**22**			**22**
21-Sep-22	0648	0742.940S	15613.800E	Anchored FAD	**35**			**35**
	0954	0748.481S	15620.990E	Seamount	**29**			**29**
22-Sep-22	1714	0748.098S	15821.176E	Anchored FAD		**1**		**1**
23-Sep-22	1715	0723.400S	15836.900E	Free school	**4**			**4**
24-Sep-22	1336	0805.280S	15753.790E	Anchored FAD	**39**			**39**
25-Sep-22	0820	0811.940S	15805.750E	Anchored FAD	**70**	**46**		**118**
	1652	0838.007S	15832.120E	Free school	**73**			**73**
26-Sep-22	0924	0859.300S	15733.850E	Anchored FAD	**115**	**5**		**120**

	1013	0900.600S	15736.480E	Free school	**266**	**15**		**281**
	1057	0902.870S	15739.430E	Anchored FAD	**705**	**30**	**1**	**736**
	1215	0902.600S	15737.800E	Free school	**44**			**44**
	1318	0907.000S	15735.030E	Free school	**200**			**200**
	1413	0909.600S	15731.230E	Free school	**104**			**104**
	1746	0852.990S	15729.100E	Free school	**155**			**155**
27-Sep-22	1545	0923.170S	15814.600E	Free school	**62**			**62**
	1737	0923.300S	15827.800E	Anchored FAD	**137**	**55**		**192**
28-Sep-22	0551	0939.700S	15924.000E	Anchored FAD	**25**			**25**
	0711	0835.300S	15921.600E	Anchored FAD	**113**	**2**		**115**
	0835	0836.300S	15913.700E	Free school	**190**	**2**	**1**	**193**
	1027	0828.500S	15910.800E	Anchored FAD	**84**	**21**		**105**
	1235	0829.450S	15920.230E	Free school	**316**			**316**
29-Sep-22	0844	0853.360S	15921.700E	Anchored FAD	**71**	**1**		**72**
	1004	0834.600S	15914.200E	Free school	**589**			**589**
	1217	0834.800S	15915.100E	Anchored FAD	**540**	**104**		**644**
30-Sep-22	1208	0830.090S	16021.550E	Seamount	**54**			**54**
01-Oct-22	0802	0923.150S	16049.900E	Free school	**823**			**823**
	1017	0925.100S	16054.800E	Free school	**293**			**293**
	1137	0924.250S	16056.370E	Free school	**240**			**240**
	1217	0924.850S	16057.680E	Free school	**47**			**47**
	1340	0932.560S	16100.900E	Free school	**510**			**510**
	1432	0935.480S	16100.280E	Free school	**242**			**242**
	1514	0934.500S	16056.180E	Free school	**268**			**268**

02-Oct-22	1243	0931.900S	16056.900E	Free school	**695**			**695**
	1353	0934.000S	16052.400E	Free school	**225**			**225**
	1433	0935.300S	16052.600E	Free school	**43**			**43**
	1455	0936.180S	16053.400E	Free school	**273**			**273**
	1525	0935.400S	16052.800E	Free school	**341**			**341**
	1727	0936.900S	16058.200E	Free school	**198**			**198**
03-Oct-22	0617	0936.000S	16103.950E	Anchored FAD	**353**			**353**
	0651	0938.080S	16102.600E	Free school	**674**			**674**
	1000	0937.500S	16100.600E	Free school	**321**			**321**
	1121	0932.014S	16058.800E	Free school	**36**			**36**
	1140	0932.270S	16059.700E	Free school	**234**			**234**
	1332	0929.800S	16047.800E	Free school	**70**			**70**
04-Oct-22	1447	0848.000S	15924.000E	Free school	**1718**			**1718**
05-Oct-22	0550	0812.000S	15805.700E	Anchored FAD	**283**	**4**		**287**
06-Oct-22	1503	0809.600S	15802.600E	Free school	**243**			**243**
	1643	0812.430S	15805.920E	Anchored FAD	**37**	**12**		**49**
07-Oct-22	0825	0821.100S	15756.920E	Free school	**414**			**414**
	1211	0806.140S	15753.820E	Anchored FAD	**9**			**9**
	1245	0805.600S	15755.760E	Free school	**306**			**306**
	1459	0759.900S	15747.850E	Anchored FAD	**173**			**173**
	1542	0757.500S	15746.040E	Free school	**440**			**440**
TOTAL					**16639**	**334**	**2**	**16977**

Appendix 2

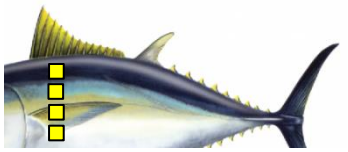
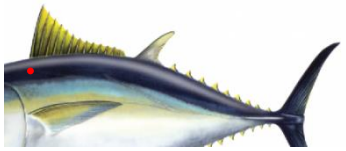
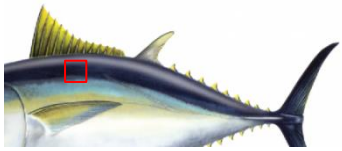
Biological sampling plan for WP6

This biological sampling plan is designed for the **WP6 pole-and-line tagging cruise** aboard NFD's Soltai 105. WP6 will focus on the archipelagic waters of the Solomon Islands' EEZ and will run during September and October 2022. This plan covers five main sampling tasks, summarised below.

6. Routine sampling (for the WCPFC Pacific Marine Specimen Bank)
7. Fatmeter measurements
8. $SrCl_2$ marking and biopsy sampling at release
9. SKJ stomach microbiome experiment
10. Among-sampler variability in DNA contamination

1. Routine sampling

Priority species	SKJ, YFT, BET. Where time permits, sample all other landed species.
No. of fish to sample	Per fishing event For each fishing event, termed a 'school' on the pole-and-line tagging cruise, sample fish not destined for tagging in the following order:- – 10 SKJ – 10 YFT – 10 BET – Remaining SKJ – Remaining YFT – Remaining BET – All other landed species If < 10 fish are landed per species, sample all fish of that species. Opportunistic sampling Sample any fish captured while trolling between fishing events. As heavy trolling gear is not available for this cruise, billfish are unlikely to be encountered. Small tunas, Spanish mackerel, and wahoo are more likely candidates.
Order of tasks	i) Record length. ii) Record whole weight (for tunas only). iii) Take Fatmeter measurement (for tunas only). iv) Collect biological samples.

Notes on length and weight measurements	– For tuna: measure fork length (i.e. upper jaw to fork in tail) to **lowest** cm, and whole weight to **nearest** 0.1 kg. – For other non-billfish species: measure fork length (i.e. upper jaw to fork in tail) to lowest cm. – For billfish: measure fork length (i.e. lower jaw to fork in tail) to lowest cm.
Notes on Fatmeter measurements 	– Take Fatmeter measurements on all tunas from which biological samples are collected. – Record 8 Fatmeter measurements per fish, 4 on each flank at the locations shown at left in yellow. – We will have two Fatmeters onboard. Use Fatmeter #1 for these measurements on biologically sampled fish, and Fatmeter #2 for measurements on fish on deck not destined for biological sampling as detailed in 2. Fatmeter measurements.
Types of biological samples and order of collection	– For tuna: biopsy punch + 1st dorsal spine + muscle + stomach + gonads + liver + otoliths. – For other non-billfish species: biopsy punch + muscle + stomach + gonads + liver + otoliths. – For marlin: biopsy punch + 1st, 2nd, 3rd, 4th dorsal spines (i.e. D1, D2, D3, D4) + muscle + stomach + gonads + liver + head *. – For swordfish (if any): biopsy punch + 2nd fin ray + muscle + stomach + gonads + liver + head *.
Notes on biopsy punch sampling 	– Sampling position = the back, just anterior and ventral to the first dorsal fin (see red dot at left – not to scale). – Use a single-use, sterile 'biopsy punch' tool to collect a tissue plug for genetics studies. – Full step-by-step procedures for taking a biopsy punch sample are set out in Appendix 1.
Notes on muscle sampling 	– Sampling position = the back, immediately ventral to the first dorsal fin (see red square at left – not to scale). – Use a knife to cut out a large (~5 × 5 × 3 cm) piece of muscle, remove the skin. – Used for muscle stable isotope studies and contaminant analyses such a mercury.

***** For marlin and swordfish head samples, no need to take the whole head, just a square section of it that contains the otoliths.

Detailed sampling procedure

Using the 'WP6 BIOLOGICAL SAMPLING FORM', for each sampled fish:-

1. Record species, length and whole weight (the latter for tunas only).
2. Record Fatmeter measurements (for tunas only).
3. Wipe fish down using single-use rag.

4. Take biopsy punch sample, and eject resulting tissue plug into a 2-mL cryovial containing RNA*later*, pre-labelled with the 'P'-number label for that fish.
5. On biological sampling form enter 'gen' followed by the sampler's name in the 'Comments' field.
6. Determine the sex (if possible).
7. Collect other samples in order specified in the table above.
8. Record the otolith code in the 'Comments' field.

Code	Description
Oto1B	Collected only 1 otolith and the otolith IS BROKEN
Oto1G	Collected only 1 otolith and the otolith is NOT BROKEN
OtoGB	Collected 2 otoliths and 1 otolith is broken
Oto2B	Collected 2 otoliths and both otoliths are broken
Oto2G	Collected 2 otoliths and the otolith are NOT BROKEN
Head	Collected the head instead of the otoliths

← Otolith codes

9. Put muscle, stomach, liver in separate small zip-locked plastic bags with P- number labels.
10. Put dorsal spine and gonads in the same small zip-locked plastic bag with P-number labels.
11. Put otoliths (as clean and dry as possible) in small microtube with P-number label.
12. Roll samples from steps 9 and 10 together and place in larger, zip-locked plastic bag (containing samples from all fish in that fishing event).
13. Place otolith microtubes in larger, zip-locked plastic bag (containing otoliths from all fish in that fishing event).
14. Place biopsy punch cryovials in 10 × 10 storage box (that holds tissue plugs from all fish in that fishing event) and into the freezer.

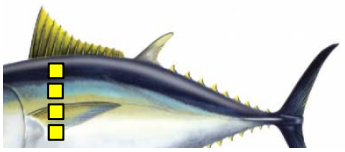
At the completion of sampling for a given fishing event:-

1. Place all samples (except otoliths) in freezer.
2. Check that all relevant information is entered on the WP6 BIOLOGICAL SAMPLING FORM.
3. Download Fatmeter data.
4. Enter all information in Excel spreadsheet.

2. Fatmeter measurements

WP6 also provides an opportunity to boost our numbers of Fatmeter measurements for the length classes of tunas captured by pole-and-line. Data on tuna fat content provides insight into the quality and quantity of prey available in the environment and on the body condition of tunas, and so represents a potentially valuable ecosystem indicator.

Our objective is to obtain accurate Fatmeter measurements on as many SKJ, YFT and BET as possible as they come aboard during a fishing event. Note that Fatmeter measurements are to be made only on fish on deck that are *not destined* for tagging, and *not destined* for biological sampling as part of 1. Routine sampling (which also incorporates Fatmeter measurements). This is so that we maximise numbers of fish measured and don't measure the same fish twice. To ensure that we can easily track the order of Fatmeter measurements recorded, we will use Fatmeter #2 for this component.

Who will do it?	A member of the science team not involved in tagging operations.
When?	During a fishing event.
Order of tasks 	As tuna not destined for tagging come onboard during a fishing event: i) Put 10 fish (per species) aside that are to be bio-sampled at the end of the event. Don't take length or Fatmeter measurements on these fish. ii) Focus only on the other fish on deck. iii) Measure fork length (i.e. upper jaw to fork in tail) to lowest cm. iv) Record 8 Fatmeter measurements per fish using Fatmeter #2, 4 on each flank at the locations shown at left in yellow.

3. $SrCl_2$ marking and biopsy sampling at release

Given the focus of WP6 on the archipelagic waters on the Solomon's EEZ, we expect higher tag recovery rate from WP6 releases compared with WP5, though time-at-liberty might be comparatively short given how the fishery operates in the area.

Good numbers of recaptures from $SrCl_2$ marked SKJ from the Solomons' EEZ will contribute important data to the ongoing SKJ otolith ageing work underway in the Indian Ocean. Additionally, taking a genetic sample from these $SrCl_2$ marked fish at release, and pushing our tag recovery network to ensure a muscle sample is collected at recovery, will provide much-needed samples for validation of the epigenetic ageing model for SKJ. This will be particularly useful if at least some of our tagged fish remain at liberty for more than six months.

In an effort to get data from these fish with longer times at liberty, our idea is to spread the sampling across all fishing events, with a particular focus on free schools for the $SrCl_2$ releases during WP6. This strategy will ensure we maximise numbers of fish marked across the full spatial area covered by the cruise, and if/when free schools are encountered, extra effort is then made to mark as many fish as possible from these events.

The intention is to dedicate one stern tagging cradle for $SrCl_2$ marking and biopsy sampling. Marion will make the call as to if a fish is in good enough condition for $SrCl_2$ marking and biopsy sampling. If it is not, a conventional yellow tag is applied.

Who will do it?	– Two science staff will be needed. – Marion is best placed to do the $SrCl_2$ marking and tagging, given her experience from WP5. – Another member of the science team will do the genetic sampling and be responsible for sample organisation and storage.

When?	During all fishing events on AFAD-associated and free schools. Particular focus on free schools, which include log and whale shark associated schools.
Order of tasks	i) A SKJ comes aboard and is placed on the cradle. ii) Measure fork length to lowest cm. iii) If fork length is between 38 and 64 cm, then, iv) Inject $SrCl_2$ (dosage tailored to fish size - see Table 1) v) Take genetic sample (in location matching that shown in 1. Routine sampling). NOTE: use WIDGET for taking samples until all WIDGET tips are used or the system proves impractical. Then use biopsy punches. vi) Insert white tag, and release. vii) Remember to fill out the Live Genetics Sampling form in conjunction with the Tag Release form
Target numbers?	We will have sufficient $SrCl_2$ solution to inject ~2000 SKJ.
Other notes	- If length isn't specified in Table 1, the injection volume for $SrCl_2$ should be rounded up. E.g. for a 38 cm SKJ, inject 2.5mL. - As our priority is on SKJ, if YFT or BET are captured in a free school event, conventionally tag them with a yellow tag and do not apply a $SrCl_2$ injection or biopsy punch sampling on these fish. - If SKJ < 38 cm or > 64 cm fork length are encountered, apply a conventional yellow tag and do not apply a $SrCl_2$ injection and do not take a genetic sample on these fish. -This is the only genetic sampling that will be done with the WIDGET. All other genetic sampling (i.e. during biosampling) will use biopsy punches.

Table 1: Dosage for SKJ with a 40mg/mL $SrCl_2$ solution

Length (cm)	Dose (mL)
38	2.5
40	2.5
43	3
45	3.5
46	4
48	4.5
50	5
51	5.5
53	6

54	6.5
55	7
57	7.5
58	8
59	8.5
60	9
61	9.5
62-64	10

Taking and recording genetic samples

We will attempt to use the 'WIDGET' for this experiment. Use WIDGET until all WIDGET tips are used or the system proves impractical. We will then use biopsy punches (see Appendix 1 for biopsy punch protocols).

Mechanics of taking a WIDGET sample

1) Clean handle by swilling the tip in a bucket of soapy water (use the genetics bucket). Do this before the first sample and between all samples.
2) Take tips provided in individual plastic baggies, open baggie and attach tip to the spring loaded WIDGET while still inside the baggie. Do not remove tip from baggie until immediately ready to use.
3) The tissue sampling position is on the back, just anterior and ventral to the first dorsal fin (see pictures in 1. Routine sampling for reference). When ready to sample a fish, press tip directly into the fish's muscle. Twist wrist once (~180 degrees) and pull directly out to remove. Do not scoop, as with the biopsy punch tool.
4) Eject tip from WIDGET into a vial of RNA*later*. Note, the spring loaded feature is pretty strong and might splash out some RNA*later*. So long as there is enough to cover the sample, it's alright for now. Top up vials if needed after the sampling event, before wrapping with parafilm.

To track and record samples

Genetic sampler will verbally record samples during the event in a voice recorder similar to the process for taggers.

1) Before a tagging event, the genetic sampler will organize prepared vials (labels starting with G001) in one working rack, plus a second empty working rack to receive filled vials. Record the event time and date and starting tag number.
2) When the tagger decides to $SrCl_2$ tag a SKJ, the genetic sampler will note the tagging block number into their recorder and move into position with a loaded WIDGET. Tagger will also confirm genetic sampling into their recorder.
3) Take the sample and eject WIDGET tip into a prepared vial.
4) Place the vial in the second (receiving) working rack and note the vial number into the recorder.
5) After the event finishes, the genetic sampler should put the rack of collected samples in the fridge/freezer until after data entry. Tagger and genetic sampler both transcribe voice recordings on respective forms (genetic sampler will use 'Live Genetic Sampling' form) and genetic sampler will corroborate notes in order to relabel vials with appropriate tag numbers.

6) Label vials (just paste a new label over the existing one), top off RNA*later* if needed, seal caps with parafilm, and move vials to a storage box.

Table 2: Example of the Live Genetics Sampling form

Live Genetics Sampling Form			
Date/ Time	Genetic Vial Number	Tagging Block Number	Tag number

4. SKJ stomach microbiome experiment

This experiment will explore the effects of time post-capture and storage on the SKJ stomach microbiome.

NOTE: As @ 05/09/22, an updated version of the protocol (that may differ slightly from the one below) has been developed and a meeting held with the WP6 crew to explain the process.

Materials/Equipment (will be ready and waiting at MRAG for collection)

- 2.5L of ThermoFisher RNA*later* or laboratory made RNA*later*
- Disposable laboratory coat/apron
- Laboratory booties
- Powderless latex or nitrile gloves
- Sterile 50mL falcon tube (x80)
- Sterile 2mL tube (x80)
- Sterile scissors (x10)
- Sterile spoons (x10)

Procedure

Preparation before sampling

- Prepare at least 60 sterile 50mL falcon tubes. Add 35mL of RNA*later* into each tube, store at room temperature.
- Prepare at least 60 sterile 2mL tubes. Add 1.5mL of RNA*later* into each tube, store at room temperature.

Sample collection

1. 50 SKJ from a single school will be collected at once. All 50 SKJ should be of similar size (i.e. within the same 5 cm length bin) in order to control the variation in size-related diet preferences.
2. The SKJ microbiome will then be sampled in five sampling events, as follows:

Sampling event	**Details**

0 hours	For 10 of the selected 50 individuals, we sample the microbiome immediately upon capture. Keep 10 other fish on deck in shade, kept wet in a basket with wet towel on top. The remaining 30 fish will be frozen at -20℃ in a brine bath.
2 hours	For the 10 individuals kept on deck, we sample the microbiome 2 hours after capture.
24 hours	For another 10 individuals kept in the brine, we sample the microbiome 24 hours after capture.
12 days	For another 10 individuals kept in the brine, we sample the microbiome 12 days after capture.
25 days	For another 10 individuals kept in the brine, we sample the microbiome 25 days after capture.

3. Record all metadata associated with each fish, including approximate length, capture date, sampling event (e.g., 0, 2, 24 hours, 12, 25 days) and assign a unique tag identifier for each fish's microbiome sample.

Sample processing

1. At each sampling event, 10 fish will be processed. For frozen fish, each fish should be defrosted at room temperature.
2. Clean the benchtop, put on gloves and prepare 20 scalpels and 10 spoons for processing.
3. Set up 11, 2mL sterile tubes with 1.5mL RNA*later* inside on a tube rack. One will be used as blank negative control, which will have 1.5mL RNA*later* and 0.5mL Milli-Q water.
4. Set up 11, 50mL sterile falcon tubes with 37.5mL RNA*later* inside on a tube rack. One will be used as blank negative control, which will have 37.5mL RNA*later* and 12.5mL Milli-Q water.
5. Mark the tubes with the sampling event and sample tag identifier on the body and the lid of the tube. Take both blank negative controls and leave them open near the sampling area. Cap the tubes at the end of sample processing.
6. Place the fish on the bench and cut open the gut cavity without puncturing the stomach or intestines. Then, use the scalpel to collect a small piece of heart tissue. Carefully place the heart tissue in the 2mL sterile tube. Cap the tube. Dispose of the scalpel.
7. Then, make a longitudinal incision of the stomach using a new sterilized scalpel.
8. Use the sterile single use plastic spoon to scrape the inner stomach lining, and carefully place the scraped tissue sample in the 50mL falcon tube.
9. Fill falcon tube with scraped stomach tissue up to the 50mL, cap the tube.
10. Discard used scalpel, spoon, and gloves.
11. Repeat 6-10 for each specimen.
12. At the end of sampling, cap two negative control tubes, store all samples and negative controls at -20℃.

5. Among-sampler variability in DNA contamination

This last experiment will test our current genetics sampling SOP's robustness to DNA cross-contamination. By engaging several of the science team and Soltai 105 crew in the biopsy punch sampling during WP6, the experiment will allow a comparison of sample cross-contamination rates when different people follow the same genetics sampling protocol, which is set out in Appendix 1.

Experimental design

Take samples as normal following the protocol in Appendix 1, with the following additional guidelines.

- All people that are willing and able should collect at least 30 genetic samples from skipjack.
- Recommendation: practice the motion for extracting samples with a biopsy punch prior to biosampling (use a sacrificial fish—does not need to be a tuna).
- Collect at least the first 10 samples in isolation from other biosamplers (or at least don't actively watch and copy each other).
- Follow the protocol to the best of YOUR interpretation. It is okay to adjust your interpretation of the protocol over time to improve your efficiency and comfort, but not simply because you observe another sampler doing it differently.

Appendix 1: Protocol for taking a biopsy punch sample

All biosampled fish should have a biopsy punch taken for genetics, as part of standard biosampling procedures. The tissue sampling position is on the back, just anterior and ventral to the first dorsal fin (see pictures in 1. Routine sampling for reference). NOTE: this does not apply to live SKJ tagged with $SrCl_2$, which will be sampled with the WIDGET.

Equipment

- Bucket of soapy water
- Optional: nitrile gloves
- Wipes, single-use and no lint/pilling
- 3-mm+ disposable biopsy punch with plunger
- 2-mL cryovials, PCR-clean with external threaded caps, filled with RNA*later* and pre-labelled

Taking a sample

- Keep hands clean and dry (no blood or fish slime). Best is to wash hands in soapy water for 5-10 seconds before handling fish and always avoid direct contact of hands with sampling site.
    - If you are not confident in your ability to avoid touching the sampling site, then wear gloves: wash GLOVED hands in soapy water (5-10 seconds) between handling fish. Airdry hands, DO NOT wipe with a reusable cloth.
- Wipe sampling location on fish with a clean, disposable wipe. Use each wipe only once. Use multiple wipes if necessary (for fish that are bigger/slimier/from the bottom of the pile), until fish skin is visibly clean and dry. Again, use the side of the wipe that your hand has NOT touched.
- Once fish is wiped and hands are clean and dry, open sterile packaging on biopsy punch tool.
- Position tool at desired sampling site, rotate and press down to cut through skin (if it's an older fish, might need to scrape off a few scales first. Can do that with the tip of the punch tool).
- With the tip of the punch tool as a pivot point, swing the tool in 2-3 wide circles to sever the subcutaneous tissue.
- 'Scoop' out the tip of the punch tool (don't simply lift out).
- Transfer sample immediately to cryovial filled with RNA*later*. Eject tissue sample using plunger function on punch tool, screw on vial lid and shake to suspend sample in solution.
    - If the chunk does not eject cleanly, you can drag the tip of the biopsy punch tool along the inside edge of the vial to coax it out.
    - If insufficient sample was extracted, you can use the same biopsy punch tool to extract more from the existing punch hole, so long as you are confident that nothing else has touched or compromised the tool. Otherwise, take more sample using a new tool.
    - Dispose of punch tool, immediately move vial out of direct sunlight/excessive heat, and into cold storage within a few hours.
    - When the sample is taken, mark 'gen' followed by the sampler's name in the comments section of the biosampling form.
    - Samples in vials can be left on ice/below 4°C for several days, -4°C for several weeks and should eventually be frozen long term at -20°C or colder.

Maintenance of the genetic toolbox

Nominate one person to be in charge of keeping genetic sampling materials prepared and organized. Ensure that:

- At least 50 additional sample vials are prepared for biosampling (filled with 1.5mL of RNA*later* and labelled to correspond with upcoming biosampling 'P'-number labels)
- At least 100 additional sample vials are prepared for $SrCl_2$ tagging (filled with **1.2mL** of RNA*later*, labelled using 'G'-number labels, starting G001)
- There are vials, Kimwipes (or other disposable wipe) and biopsy punches immediately available for at least 50 samples in the on-deck genetics box. No water or other solutions have splashed on the Kimwipes
- RNA*later* isn't left unnecessarily baking in the sun for hours on end (e.g. move biosampling vials out of the on-deck box and in the shade while doing hours of midday $SrCl_2$ tagging)
- WIDGET handle is cleaned in soapy water and free of visible contaminants
- Working racks are clean of salt spray, fishy liquids, etc
- Handwashing bucket is clean and not borrowed for other tasks
- Once samples are taken, the caps are sealed with parafilm and vials are appropriately stored. Additionally, for $SrCl_2$ samples, RNA*later* has been topped off and labels updated with tag numbers. (On the GL4, I keep one storage box for each experiment in the galley freezer to collect vials immediately after sampling finishes, and move it to the blast freezer once it is full. Before putting in long term storage I also tape the lid shut with masking tape and label it with the range of vial numbers inside.)