PACIFIC TUNA TAGGING PROJECT

Phase 2 (Central Pacific)

Cruise CP-14, 15th August to 2nd October 2020

SUMMARY REPORT

Jeff Muir, Marion Boutigny, Giulia Anderson, West Kekoa Seward and Alex Filous

INTRODUCTION

This report summarizes activities during the 49 days of a fourteenth Central Pacific research cruise (CP-14), on the San Diego-based FV Gutsy Lady 4. Due to the COVID 19 pandemic, the cruise was designed with a (mostly) Hawaii based science crew sampling in a geographic area suited to a Hawaii arrival and departure that maximized working days at sea (vs. steaming) and involved no intermediate Port stops for reprovisioning or crew change. CP-14 was designed to augment data collection for studies on tuna movements, exploitation rates and fish aggregation device (FAD) association dynamics in the WCPO. It was the first major tagging event to incorporate significant numbers of drifting FADs (dFADs) in the geographical area as part of its sampling design. The geographic area of CP-14 were 10°N-4°S, 175°W-150°W, in international waters, as well as the Line Islands as well as the Phoenix Islands Protected Area (PIPA) Within the EEZ of Kiribati. Generally, the cruise plan originating from Honolulu was to steam to around 8°N/155°W, begin fishing on dFADs while visiting TAO moorings along the 155° meridian to the equator, and then continue west, fishing along the way on dFADs, and return north on the 170°W meridian and then back to Honolulu (**Figure 1**).

Location of FADs was made possible by the cooperation between SPC, Cape Fisheries and the U.S. Tuna Group.

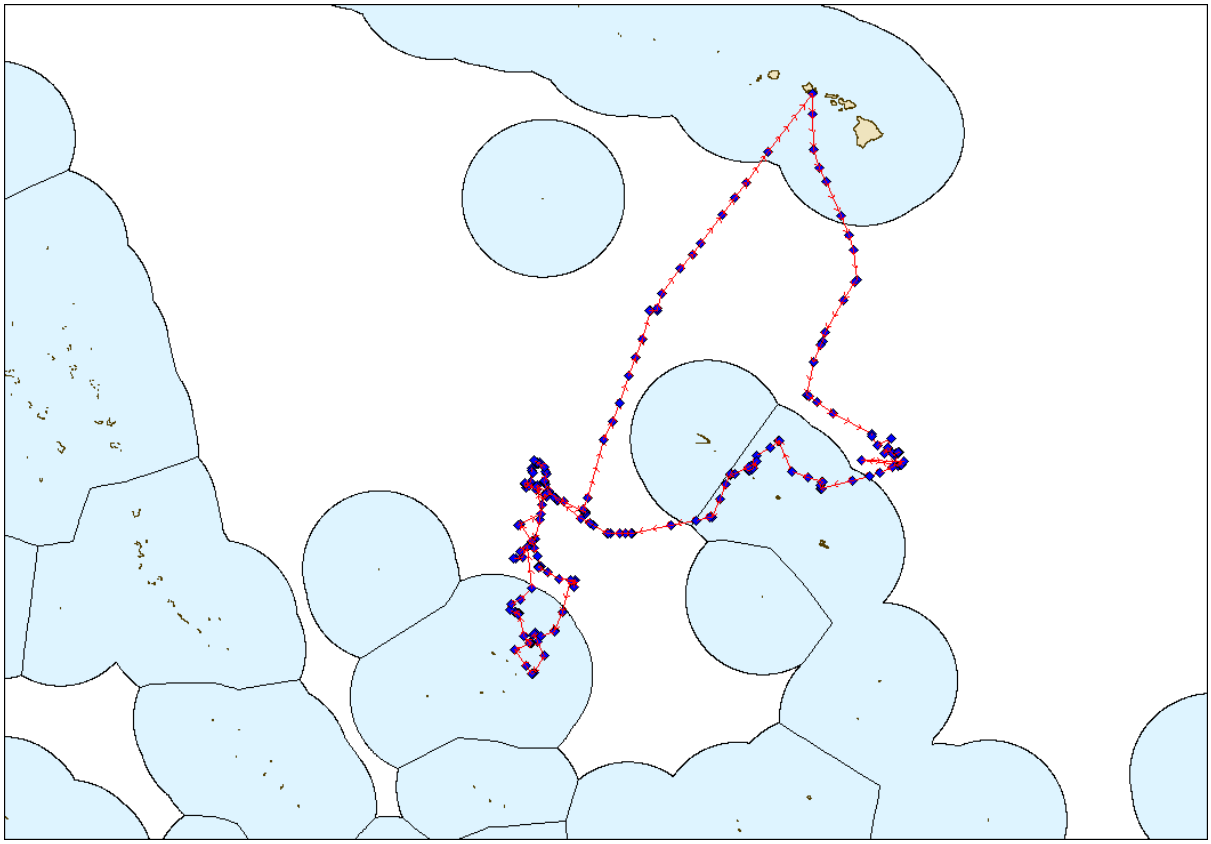


Figure 1: Forty-nine day cruise track (red arrows) and daily positions (blue squares) of CP-14.

Crew and scientific personnel onboard Gutsy Lady 4 during CP-14 are listed in **Table 1**.

Table 1: Personnel onboard Pacific Sunrise during CP-14

Name	Title/affiliation	Nationality
Tim Jones	Captain	U.S.
Ben Stephens	First mate/engineer	U.S.
Jeff Muir	Cruise Leader/contractor	U.S.
Marion Boutigny	Contractor	France
Giulia Anderson	Scientist/ SPC	U.S.
West Kekoa Seward	Contractor	U.S.
Alex Filous	Contractor	U.S.
Ali Saputra	Crew/Bosun	Indonesia
Nurrofik	Crew	Indonesia
Jaenel Abidin	Crew	Indonesia

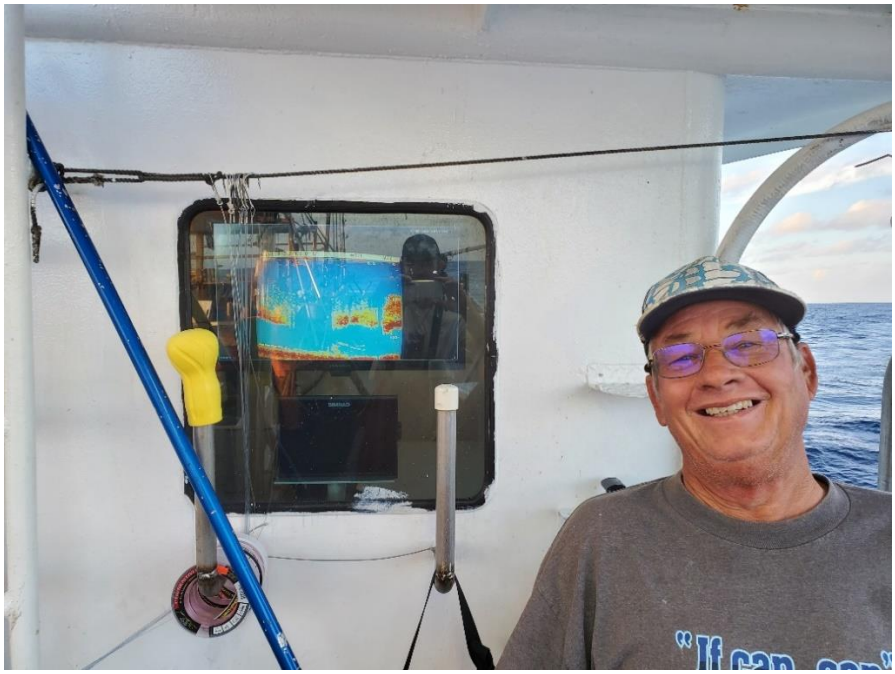
GENERAL DESCRIPTION OF VESSEL

The FV Gutsy Lady 4 (named hereafter GL4) is a 30 meter steel vessel (**Picture 1**) previously outfitted for shrimp trawling in the Gulf of Mexico. It is now equipped with longline gear and used for fishing pelagic fish (mainly tuna, with bigeye as the main target) in the Hawaiian EEZ. The vessel is fitted with two 600hp Cummins engines, two 70 KVA Cummins generators, and one water-maker (80 l/h). The

vessel is fully equipped with Furuno electronics including 3 VHF and 1 SSB radios, radar and dual frequency sounders (FCV 295 + 3KW transducer), autopilot, AIS, a vessel monitoring system (CLS), 2 water temperature gauges, a longline LP system, one desktop computer for navigation (HighPlot, custom-made by an ex-fisherman) and the OrbMap oceanography information package. GL4 is also equipped with an Iridium satphone linked with Skyfile software for email communication. The vessel is owned by Tim Jones (**Picture 2**) and the Hayworth family in San Diego. Its current home port is San Diego, CA, with fishing time split between Hawaii and the west-coast of the USA..

Picture 1: FV Gutsy Lady 4 at Kewalo Basin, Honolulu, Hawaii 15th August 2020

Prior to CP-14 departure, the GL4 was outfitted with a Fleet One satellite communication system coupled with an "Oceanbox" data compression server (Thalos). This system was used in CP-12 and CP-13, and then mothballed for 2 years in the interim between cruises. Upon reinstallation of the system, several issues were discovered including a faulty antennae cable and firmware/software issues. Thalos customer support was extremely helpful and capable and worked remotely with the Cruise Leader to resolve multiple issues and get the system fully operational before departure. The system was used for buoy management via Satlink ELB software and for scientific and contracted staff email.



Picture 2: Captain Tim Robert Jones and a big jag of bigeye (background on screen)

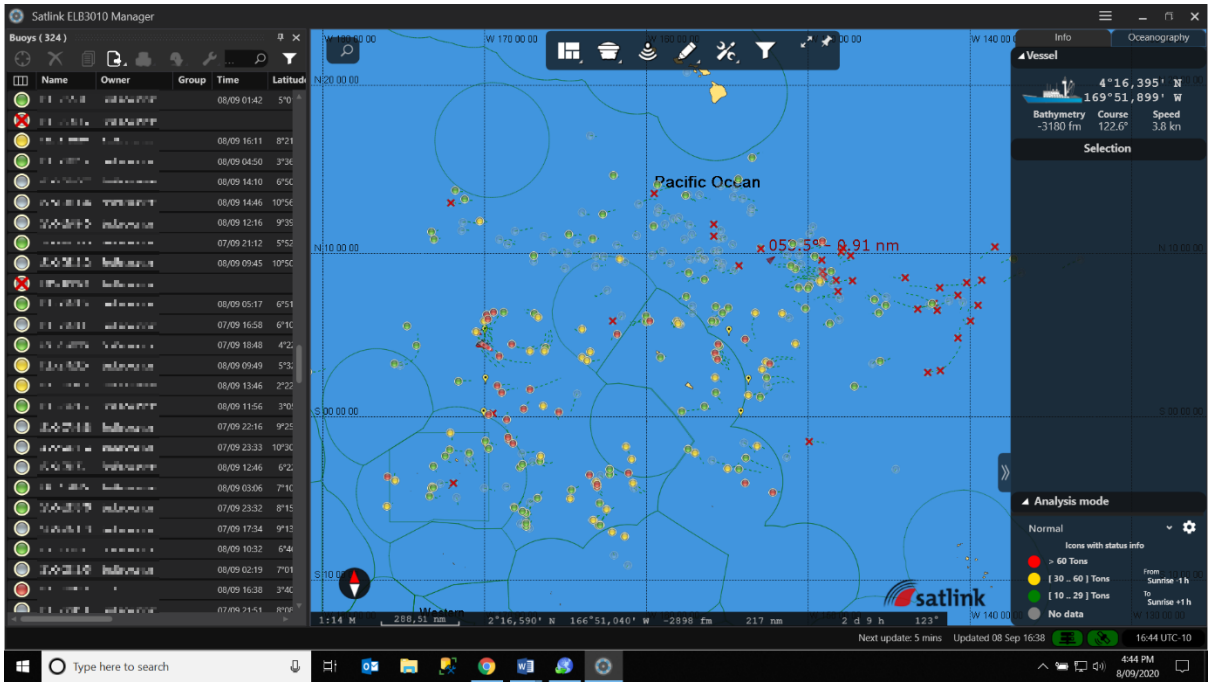
Complete boat specifications are detailed in **Appendix 1**.

The operational range of GL4 is over 10,000 nm and 60 days at 8 knots with a total fuel tank capacity of 110,000 litres. The boat also has a fresh water tank of 30 m^3 capacity and a 2 tons/day capacity ice-maker. The fish hold is divided into two parts, one dedicated to preserve fish in ice (about 22 ton capacity) and one freezer compartment, mainly used to store frozen bait (about 15 tons).

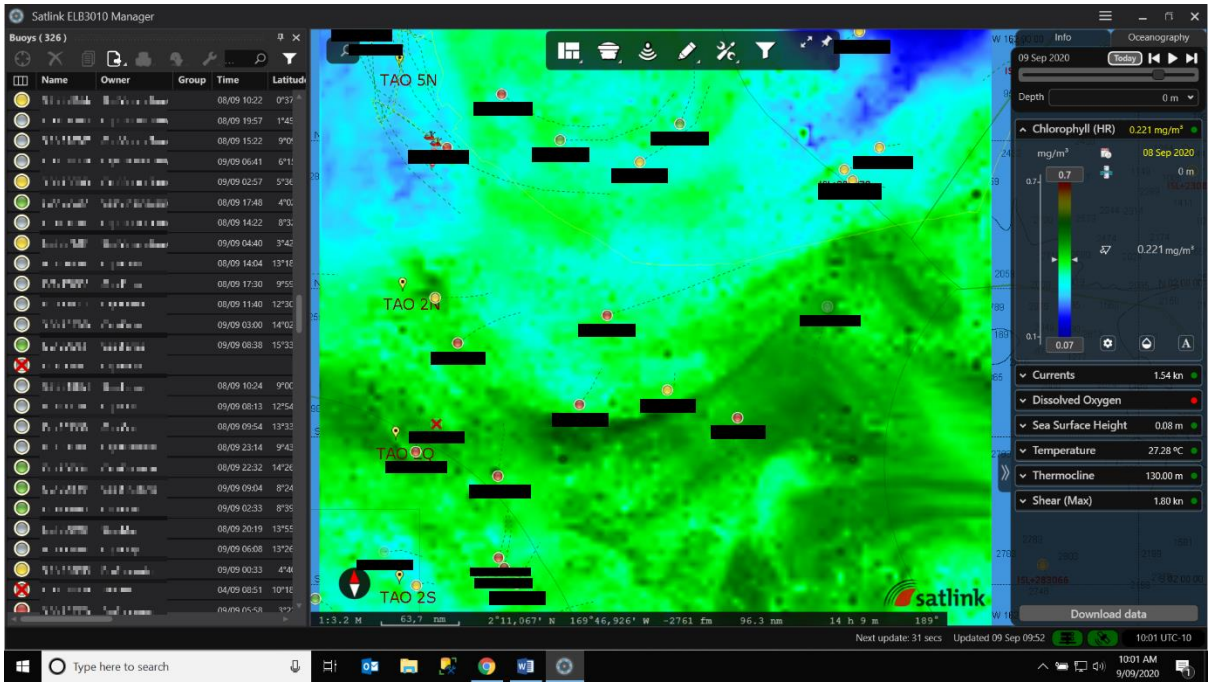
Access to dFADs and satellite buoy data information used during the cruise

Cape Fisheries (formerly Trimarine) and the US Pacific Tuna Group provided full access to dFADs owned by them, all of which are equipped with Satlink ISL, SLX, and ISD satellite buoys, in the areas that the tagging vessel operated during the cruise. Both companies agreed to share their buoys between 15 August and 25 September, with this agreement made directly between SPC and Cape Fisheries, and via Satlink with the vessel owners of the USPTG. Cape Fisheries allowed further access to buoys above 8N for the remainder of the cruise, as this was well out of their operating area and would not interfere with the 1st October opening of FAD fishing. Both companies had geographic fences upon which their dFADs would appear and disappear when crossed (dFADs crossing into the WCPO over the 150W meridian were turned on, for example). The maximum number of dFADs that were shared was 341, and most of the time was >300 (**Picture 3**). A total of 32 different dFADs were visited and fished (See **Figure 3** for an overview of dFAD locations).

Satlink ELB3010 Manager software was used for buoy management and querying. The program also allows the user to download oceanographic data to overlay on buoy location maps (**Picture 4**). This was quite useful when making large scale decisions about where to direct fishing effort, since the information is relevant to aggregation catchability (via chlorophyll boundaries, SST and current) and steaming plans (via buoy drift and current speeds).



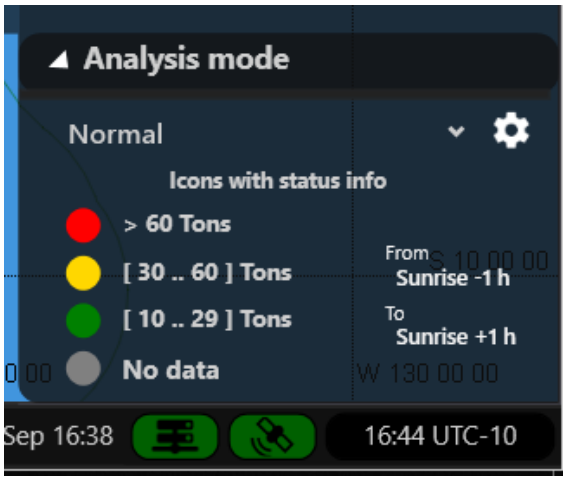
Picture 3: Screen display of Satlink ELB3010 Manager dFADs in CP-14 study area.



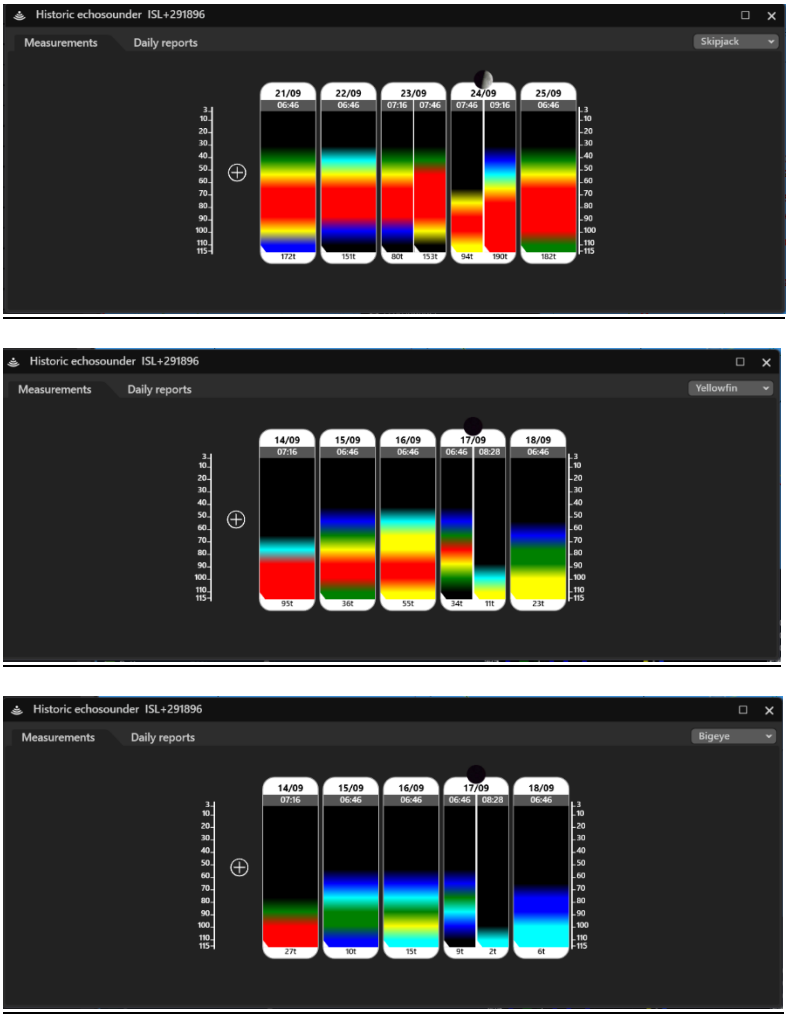
Picture 4: Example of ELB3010 Oceanographic data. Chlorophyll layer shown, note position of dFADs along boundaries of higher concentrations of chlorophyll.

Each buoy utilized echosounder data collected at different times of the day (depending on the model of buoy) to estimate the tonnage of fish, and further categorized by species. A four color system (**Picture 5**) was used to differentiate tonnage estimates to make planning easier. Tonnage estimates (**Picture 6** as an example) seemed to be inaccurate, usually overestimating total tonnage probably because of the presence of larger bigeye (with larger swim bladders). However, there was no way to empirically confirm this with the resources available on the GL4. It was useful to use the tonnage estimates more as a total biomass indication, rather than rely on it to make planning decisions based

on how many tons of bigeye or yellowfin were predicted. This seemed to work well for the purposes of a hook and line tagging trip.



Picture 5: Color-coded tonnage estimates on ELB3010 software.



Picture 6: Tonnage estimates for SKJ, YFT and BET for dFAD ISL+291896

FISHING GEAR

For this tagging cruise, the vessel was fitted with 8 “danglers”. This gear consists of stainless steel or aluminum davits which extend at right angles from the hull for 2 meters and deploy two short trolling lines skipping at the surface. This technique has been successfully used during the thirteen previous CP cruises as well as in Hawaii for other tagging programs. Initially developed for commercial fishing at offshore seamount and FAD tuna aggregations in Hawaii, it is still used in Hawaii by a handful of commercial fishermen.

Five danglers were placed on the starboard side and 3 on the port side. The troll lines hanging from the danglers consisted of a 2m length of 6mm rope spliced with loops at both ends, to which an 80cm length of 2mm monofilament line was fitted with a variety of trolling lures and a 7/0 Mustad galvanized barbless hook.

Three troll lines were also deployed on hydraulic reels attached to the stern of the vessel. The lines consisted of 400 lbs monofilament line, to which a 5m length of 2mm monofilament line was attached and rigged with a trolling lure and a 7/0 Mustad galvanized barbless hook.

Jigging landed a large proportion (51%, see Table2) of the fish tagged during CP-14, and nearly 100% (1 YFT was caught on trolling gear between stations and tagged with an MK9) of the fish that were implanted with archival and sonic tags. The same pattern was noted on previous cruises (mostly CP’s 11-13) but was capitalized on during CP-14. When conditions allowed, 4 rods and 2 handlines were jigging simultaneously, and this resulted in multiple hook ups for most of the duration of the jigging sessions. Timing of jigging sessions was also critical; 02:00 seemed to be a good start time as it allowed enough time before daylight to have a decent amount of effort, but at the same time not so early that it prematurely wore the crew down. Jigging after daylight was difficult during CP-14; wahoo plagued almost every dFAD visited and were quite adept at clipping off jigs and freshly tied fluorocarbon topshots.

Picture 8: jigging team, and then some others not so busy…

TAGGING OPERATIONS

Four tagging stations were set up on the deck of the vessel. Three cradles were dedicated to conventional tagging (**Picture 9**) and were of the same design to those previously used for pole-and-line tagging. One cradle was placed at the stern of the vessel while the other two were positioned on the starboard side. The fourth cradle was set up specifically for archival/sonic tagging and supplied with a saltwater hose for irrigating the fish during surgery (**Picture 10**). This tagging station was also used to deploy the sonic tags in the array experiment. The archival cradle was placed in a central location on the deck. All cradles were marked with one cm graduations from 30cm to 120cm.

Data recording

Each tagger was equipped with a digital voice recorder enclosed in a waterproof sleeve. The first and last tag in each new block was read out before commencing tagging, and tag numbers were intermittently recorded and checked. After each fish was tagged, its length was recorded from the graduations on the cradles. Data were later transcribed onto hard copy release log sheets at the end of each tagging session. Data were subsequently entered into the Microsoft SQL Server data base "TagDager".

Picture 9: Conventional tagging cradle with a 20 kg bigeye tuna ready to be tagged and biopsy sampled

FISH TAGGING DETAILS

Table 2 summarizes numbers of fish caught with each gear type and average number of tags per event.

Table 2: numbers of tags deployed by each gear type, and numbers per event.

	Jig	Troll
Total Tagged	3262	3125
No. per Event	74.1	65.1
No. Events	44	48

Table 3 summarizes the number of fish tagged per tag type and per species.

Table 3: Numbers of tags deployed by tag type and species. Others include oceanic whitetip sharks and blue marlin.

Tag type	BET	YFT	SKJ	others	Total
Sonic	32	-	-	-	**32**
Archival	44	20	-	-	**64**
Satellite (miniPAT)	-	-	-	8	**8**
Conventional W13	155	89	14	-	**258**
Conventional Y13	4087	1642	304	-	**6033**
Total fish tagged	**4318**	**1751**	**318**	**8**	**6395**

Figure 2 details the length frequency distribution of all released tropical tunas during CP1-14. Note the bimodal nature of bigeye releases, with the typical juvenile fish in th 42cm peak, and then again a smaller peak at 94cm.

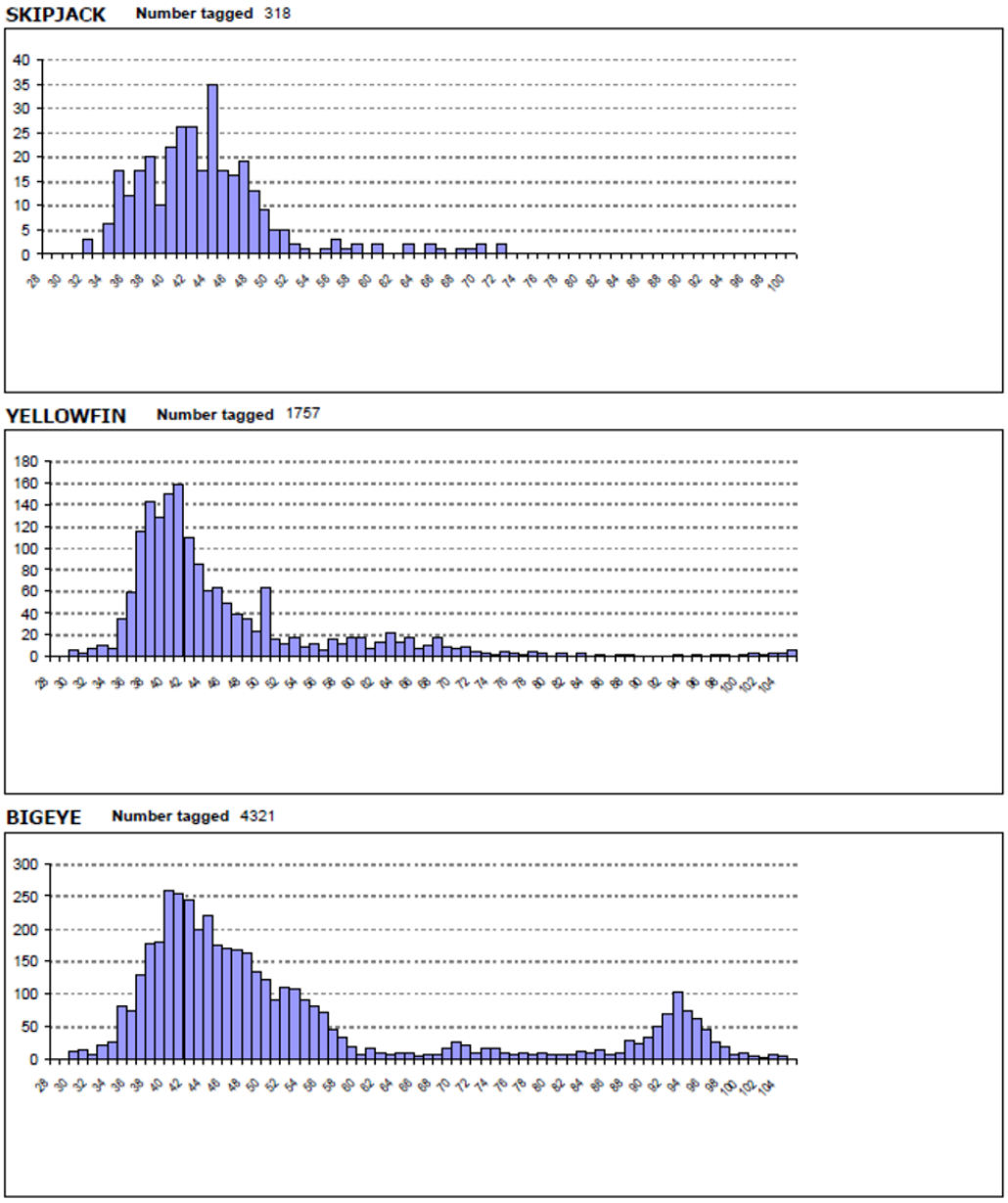


Figure 2: Length frequencies of releases by species, all tag types.

Conventional tagging

Conventional tagging (CT) uses the 13cm yellow dart tag manufactured by Hallprint Ltd. After checking if fish did not present any severe injuries[1], the tag was inserted between the pterygiophores of the second dorsal fin using a sharp stainless steel applicator tube. Used applicators were collected and immersed in a bucket containing a solution of fresh water and bleach, rinsed in fresh water and dried for re-use. Prior to each tagging operation, tags were placed inside the applicators and mounted in numbered tagging blocks each holding 100 loaded applicators. There were eleven 100-tag blocks in total.

[1] Typical injuries, incurred by large hooks and the shock/trauma of hookset, included mouth/lower jaw damage, eye damage (from inside the mouth cavity) and bleeding from various locations, and ranging from superficial to heavy. Bites from cookie cutter sharks and wounds from sharks and billfish were also noted.

A total of 6387 tropical tunas were tagged and released during the cruise, comprised of 4318 bigeye (68%), 1751 yellowfin tuna (27%) and 318 skipjack (5%). Their size distributions are shown in **Figure 2.** The spatial distribution of all tuna tag releases is shown in **Figure 3**.

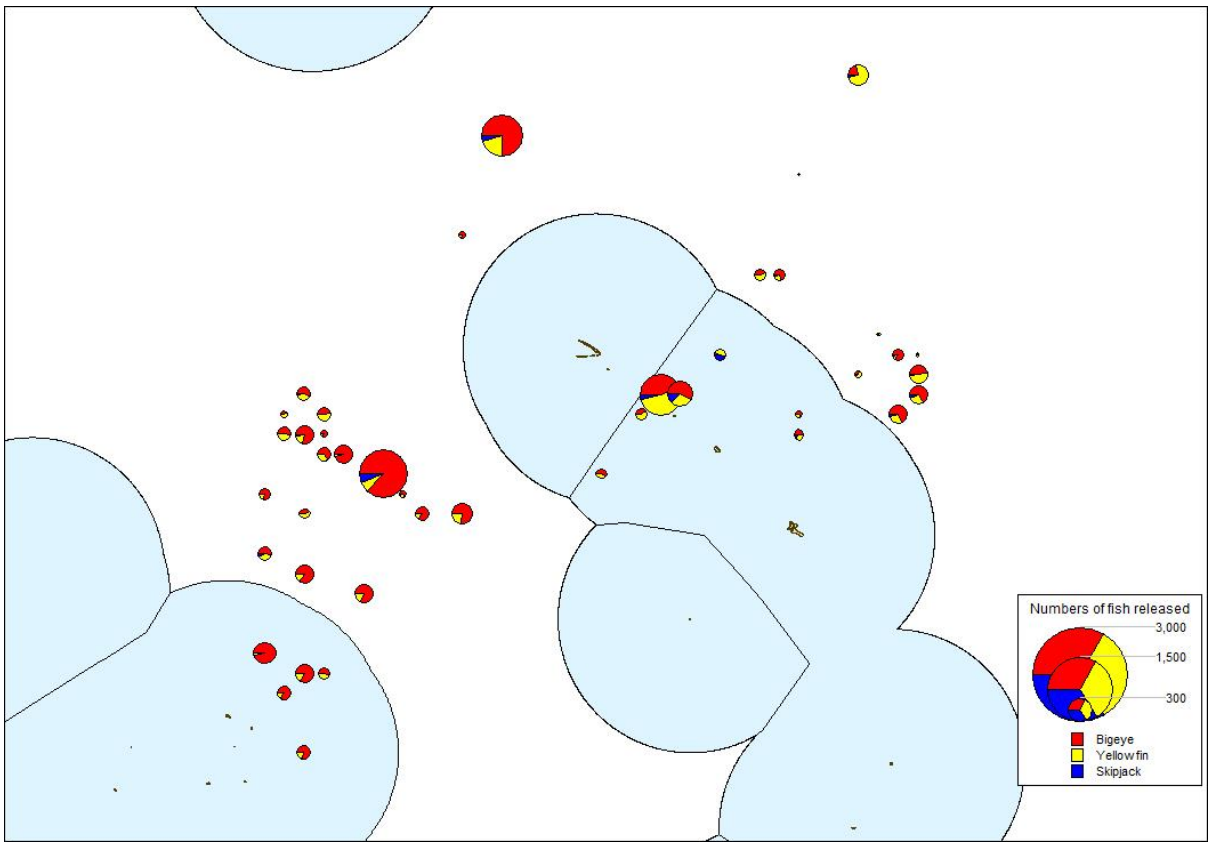


Figure 3: Spatial distribution of all releases during CP-14.

Archival tagging

Fifty Wildlife Computers MK9, and 14 Lotek LTD2310 archival tags were available for deployment. All tags were deployed; 44 in bigeye tuna, and 20 on yellowfin. All tags were configured to sample all likely depths, sea and internal fish temperatures and light intensity every 30 seconds. Archival tagged tuna were externally marked with an orange 13 cm conventional tag. Suitable sized tuna (generally > 55 cm for MK9 and > 45 cm for LAT2810, see the length frequencies (**Figure 4**) for further details) were placed belly up on the V-shaped central tagging cradle, the eye covered with a synthetic chamois and irrigated via the mouth by a seawater hose. All archival tags were implanted into the peritoneal cavity and secured with one or two sutures (**Picture 10**).

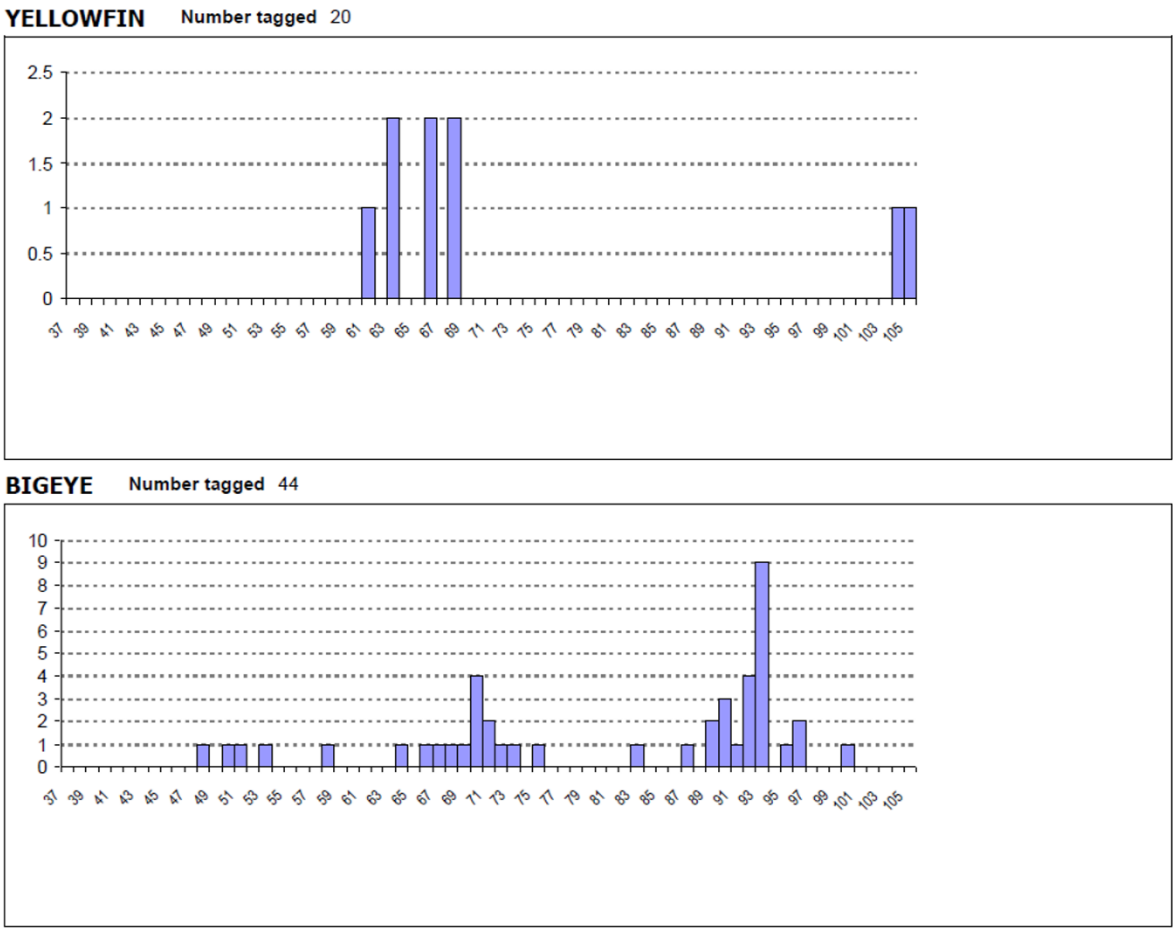


Figure 4: Length frequencies of yellowfin and bigeye tuna implanted with archival tags.

Picture 10: Archival tagging cradle

Acoustic Tagging

Acoustic tagging was a secondary objective of CP-14 after the redesign of the cruise plan due to the COVID 19 pandemic. Following some range testing in Hawaii of the Lotek and Vemco VR2W receivers, and given the interest in deploying acoustic tags and an array of receivers to drift and record school movement around the dFAD, the following objectives were identified and tested:

1. Deploy a combination of Vemco and Lotek acoustic tags, both with 69kHz frequency and the Lotek having a second 76Khz signal , in BET at a dFAD.
2. Deploy an array with the vessel's longline equipment and attempt to let it drift with the dFAD for 24 or more hours
3. Deploy VR2W and Lotek WHS acoustic receivers on the ends of the array and on the dFAD itself to collect presence/absence data for tagged fish on the dFAD
4. Ascertain each receiver's ability to detect the other manufacturer's acoustic tag simultaneously

Figure 5 shows the length frequencies of the different species implanted with acoustic tags.

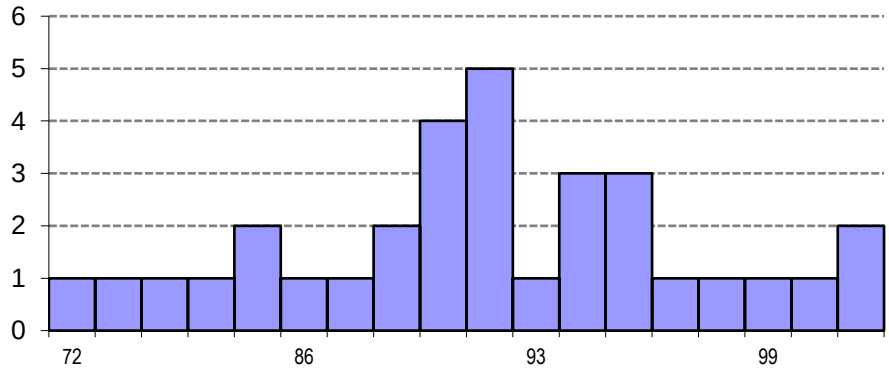


Figure 5: the length frequencies of the different species implanted with acoustic tags.

Table 4 summarizes the number of acoustic tags implanted per species and per receiver.

While working in PIPA on 17 September, a dFAD was identified as a useful place to perform the acoustic array experiment. Low (below 1kt) current and light wind were observed, and the fish in the aggregation bit well on jigs early that morning (143 CT deployed, 97% bigeye) with a low incidence of shark depredation. A single Vemco VR2W acoustic receiver was attached to the dFAD using a 10m rope, and then fishing commenced shortly before midnight on the 17th.

After surgically implanting 32 bigeye with acoustic tags (22 Lotek, 10 Vemco) (**Figure 5**) before daylight on 18 September, the equipment array was then deployed from the GL4 in the following manner: the vessel's longline spool and line shooter was used to lay a 1.6nm line parallel to prevailing current, with no hooks and 4 floats spaced evenly along the line. At one end of the line, a 3m parachute was deployed to catch the current. On the other, approximately $1m^3$ of derelict net and a 5kg piece of chain was used as an improvised drogue in an attempt to keep the line as straight as possible and in it's original length, and also mimic the drift of the tail of a dFAD. Acoustic receivers (Vemco VR2W, and Lotek WHS) as well as Satlink ISD beacons, a large float buoy, and a locator buoy were deployed as depicted in **Figure 6**. One line on each side of the dFAD was deployed. Immediately after deployment, the array was abandoned and monitored from 10nm away, to avoid interference and potential influence of the aggregation before retrieval 24 hours later. Upon retrieving the gear, the receiver on the dFAD was switched for another VR2W+Lotek WHS unit, which was abandoned on the station in hopes of recovery by the buoy's owner.

The downloaded receiver confirmed the presence of 20 of the 32 transmitters (presumably in the tagged animal and not a shark) at approximately 09:00hrs (**Figure 7**).[2] This initial result of presence and absence of tagged bigeye at the dFAD was also a useful confirmation that these larger bigeye handled capture, tagging, and release, and that survivorship from the process may be quite good; this can eliminate at least some doubt of post-release mortality for CP-14 releases of larger fish.

[2] Five of the Lotek transmitters were intermittent during testing pre-deployment. These tags likely did not function once deployed and should be excluded from any analysis.

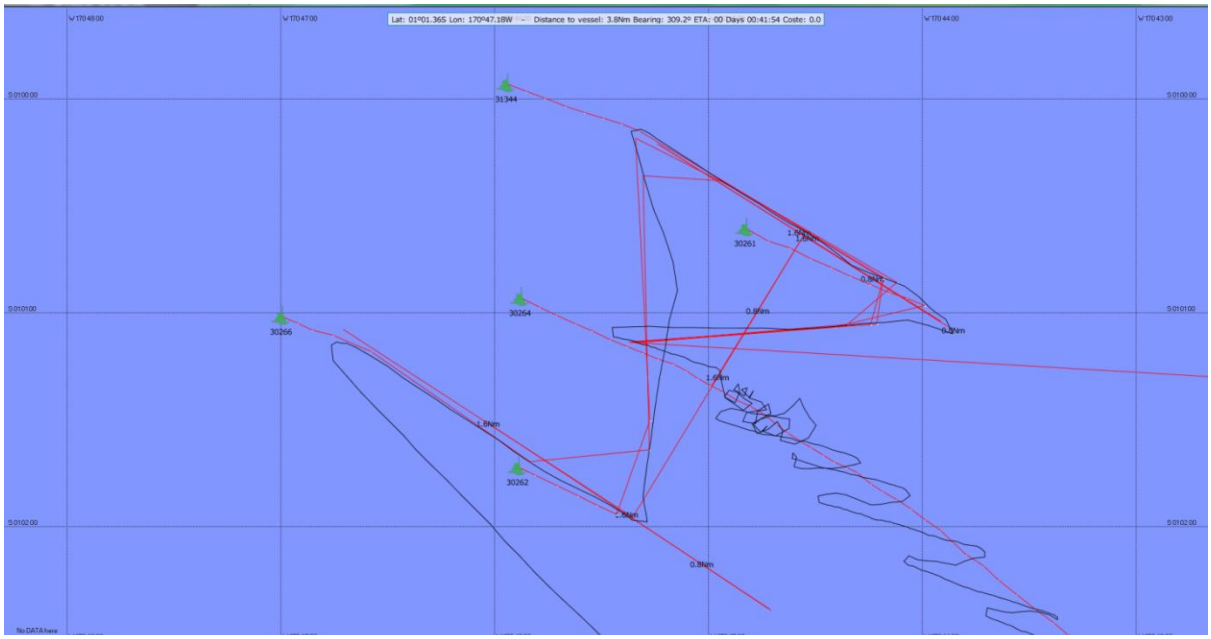


Figure 6: Initial set of drifting array and GL4 marker buoys (green icons). Black line is vessel track.

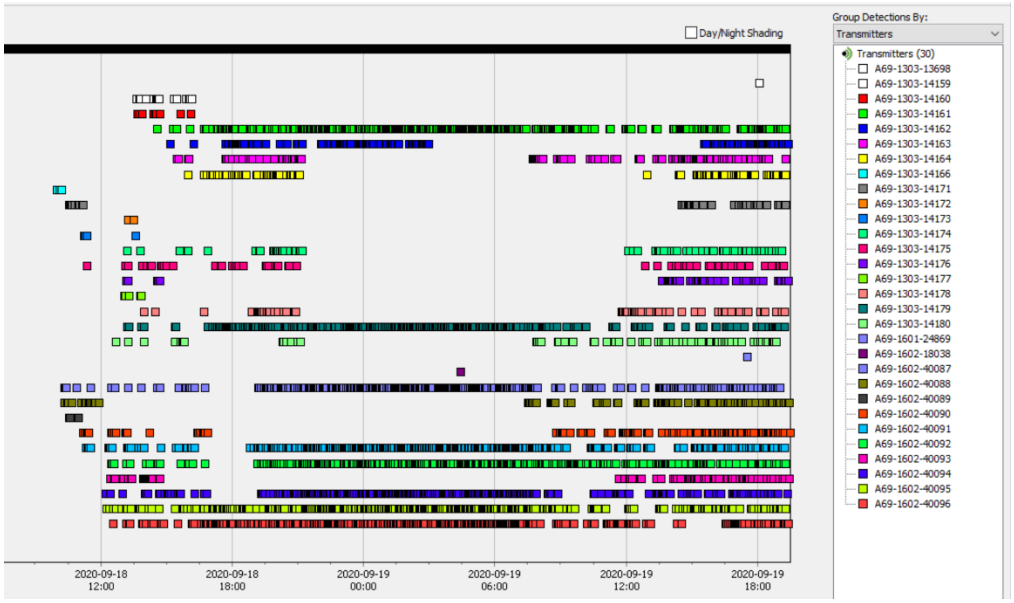


Figure 7: Abscissa plot of transmitters deployed in bigeye around dFAD up to 32hrs after deployment.

Strontium Chloride

During the cruise, 272 tagged fish (fig. xx- need to generate LF plots for SrCl fish) were injected with strontium chloride (SrCl) prior to release (258 white 13cm conventional tags and 14 archival tags). SrCl marks the otolith of the fish the moment it has been tagged. Once the fish is captured by a fishing vessel, an attempt is made at recovering the intact carcass, and the otoliths are extracted from the specimen and sent to SPC for analysis. Growth between the day a fish was tagged and the day it was recapture are inferred from the position of the SrCl mark on the otolith. On this cruise, SrCl was injected into a subset of both conventional tagged and archival tagged fish.

For a conventional tagging release, the fish is measured, injected with SrCl anterior to the first dorsal spine and above the bloodline (**Picture 11**), and tagged with a white tag. The volume of each injection depends on the size of the fish, up to 10 injections of 5ml. For example, a 50 cm BET receives 5ml; while a 70 cm BET receives approximately 15 ml. SrCl is injected using a small pistol connected to a 500ml glass bottle by a plastic tube. The pistol has a needle and a 5ml receptacle that allow to inject 0,5 ml to 5ml in the fish. Consequently, a 50 cm BET requires one injection, while a 70 cm BET needs 3 injections.

For archival tagging, the fish undergoes surgery, is measured, and has SrCl is injected at the same location as a injected for conventionally tagged fish. A fish released with both archival tags and SrCl injections is especially informative. However, fish selected for AT surgery tend to be 70+ cm up to 100+ cm, which means fish require 15-47 ml of SrCl. There were only 3 litres of solution available on board CP14, which needed to be distributed between AT and CT fish. In addition, there is a possibility the added cradle time required for 3-10 injections will slow down release time resulting in increased mortality. Consequently, it was decided to pursue quantity instead of quality, and focus on conventional tagging for SrCl. Only 14 AT fish were tagged with SrCl on CP-14.

Central Pacific cruises are perfect for SrCl injections because diverse protocols are used, and the fishing occurs at a relatively slow rate compared to pole and line tagging. However, with only 3 litres of solution available, we had to slow down the injections and ran out early. We recommend that the next cruise have 5 litres of SrCl solution.

Picture 11: SrCl injection.

BIOSAMPLING

Initial intentions were to biosample all fish that came onboard, if possible. The extensive number of mahimahi and wahoo caught early in the trip eventually forced a revision of the plan to sample only tunas and exceptional species. In total, 528 fish were biosampled, including 339 tuna (**Table 5**).

Thirty biosampled bigeye specimens were also repurposed for a genetic experiment on the impacts of sampling protocols on rate of sample cross contamination. Samples taken for that study will be further processed in a genetics-specialized lab and results returned promptly.

Table 5: Species and size class of biologically sampled specimens

Species	Length (cm)					Total
	>30	31-50	51-70	71-90	91+	
BET	0	113	33	10	28	184
YFT	1	110	10	4	5	130
SKJ	0	18	5	2		25
DOL	0	0	0	35	18	53
WAH	0	0	2	38	11	51
BUM	0	0	0	0	6	6
CFW	7	0	0	0	0	7
CNT	0	1	0	0	0	1
FAL	0	0	6	5	39	50
GBA	0	0	0	0	1	1
KAW	0	1	0	0	0	1
OCS	0	0	0	0	5	5
RRU	0	8	3	2	1	14

GENETICS

Extra protocols were developed explicitly for CP14 to facilitate the increasing application of genetic analyses in fisheries science. Genetic samples were taken from biosampled fish of all species, and from a subset of conventionally tagged tuna prior to release.

Genetic additions to biosampling

In addition to standard biosampling practices, specimens with labels P1450-P1500, P1550-P1594, P3150-P3300, and P3350-P3500 had an additional tissue sample taken explicitly for genetic analysis. Prior to biosampling, a small skin area was wiped with diluted bleach until clear of visible blood and mucus, or for at least ten seconds. Tissue samples were taken with single use, sterile 3mm biopsy punches with plunger. Multiple biopsies were taken until sufficient tissue was collected for two or more analytical events and stored in a saline solution equivalent to RNA*Later* prior to freezing.

Genetic samples were not taken for the first day of fishing. Consequently, the distribution of fish species and size classes is slightly revised from biosampling statistics (**Table 6**).

Table 6: Species and size class of biosampled specimens also sampled for genetics

Species	Length (cm)					Total
	>30	31-50	51-70	71-90	91+	
BET	0	108	33	10	28	179
YFT	1	101	9	4	4	119
SKJ	0	18	4	2	0	24
DOL	0	0	0	29	17	46
WAH	0	0	2	38	8	48
BUM	0	0	0	0	6	6
CFW	7	0	0	0	0	7
CNT	0	0	0	0	0	0
FAL	0	0	6	5	39	50
GBA	0	0	0	0	1	1
KAW	0	1	0	0	0	1
OCS	0	0	0	0	5	5
RRU	0	5	3	0	1	9

Genetic sampling via live biopsy

The potential for taking genetic samples from conventionally tagged fish was explored using a protocol that evolved onboard. A description of the most recommended adaptation is available in Appendix 2. A variety of factors influenced revisions, including the number and rate of fish caught jigging compared to dangling, fish size, and sampler proficiency with the biopsy punch tool. Key observations include: 1) it is far more feasible to sample fish during jigging, rather than dangling; 2) the use of a single-use wipe and 3 mm punch biopsy tool is insufficient to clean and consistently puncture the skin of large tunas; 3) it is much easier to sample fish in the tagging cradle than on the ground (although there is a confounding factor that large fish with thicker skins were most likely to be sampled on the ground); and 4) it is possible but not assured to collect sufficient tissue using a 3mm punch biopsy tool, regardless of fish size.

Out of 347 attempted live biopsies, 245 produced ample tissue volume for at least one genetic sequencing effort (**Table 7**). Another 56 samples are of sufficient volume for one genetic sequencing effort (**Table 8**).

Table 7: Species and size class of specimens sampled via live biopsy that produced adequate tissue volume

Species	Length (cm)				Total
	31-50	51-70	71-90	91+	
BET	95	39	27	41	202
YFT	39	1	0	3	43

Table 8: Species and size class of specimens sampled via live biopsy, including those that produced marginally acceptable tissue volume

Species	Length (cm)				Total
	31-50	51-70	71-90	91+	
BET	115	41	31	51	238
YFT	52	2	0	9	63

Shark fin clips

Live tissue biopsies were intended to also include opportunistic sampling of sharks. The punch biopsy tools, however, proved insufficient for puncturing shark skin. The protocol was revised promptly to taking fin clips using a gloved hand and knife sterilized in diluted bleach. Samples were variously stored in vials of RNAlater or sterile WhirlPak baggies and frozen, depending on resource availability. Five oceanic white tip and 50 silky sharks were sampled in total.

CONCLUSIONS

Needless to say, the fact that CP-14 even occurred in the midst of a global pandemic was remarkable. Every aspect of planning, procuring for, and staffing the cruise was complicated by supply chain issues, flight delays, and other pandemic related problems that we are all well aware of now. There was also a great (and completely rational) fear of someone on the vessel contracting COVID 19 and infecting others on the vessel after departure, and COVID testing was conducted on the entire crew prior to departure. Despite all of this, and due to an enormous team effort in Noumea and Honolulu, everything managed to come together and the cruise departed on time without issues. This in itself was already a victory in 2020.

For the last several CP cruises (CP-10 through CP-13), Cape Fisheries (formerly Trimarine) provided dFAD access for the trips. In addition to this, for CP-14, a deal was brokered through Satlink with the U.S. Tuna Group, a conglomerate of independently owned U.S. flagged vessels. They agreed to share their dFADs within the geographic boundaries of the cruise area for most of the duration of the trip nearly quadrupling the number of available dFADs for tagging and sampling. As a result, most fishing days began on a large aggregation, which already tipped the chances of success in the trip's favor. Including still more fleets in these buoy-sharing programs would further increase the chance of the success of future cruises.

The staffing of CP-14 was a unique blend of highly adept fishermen from Hawaii and skilled scientific staff. Furthermore, the crew on the vessel were already trained in Hawaiian-style tuna handline fishing, which made a notable difference in the catch rate and resulting numbers of tagged fish.

Another benefit of having a larger, skilled team is the ability to accomplish multiple non-tagging objectives effectively and in parallel to tagging operations. The number of fish biosampled during CP-14, 528, far exceeded any other sampling effort during any other CP cruise. In addition to biosampling, the opportunity to take live biopsy samples, as well as complete sampling for the cross-contamination study were capitalized on and can now inform future sampling efforts of this nature.

Jigging seems to have become a crucial part of CP cruises not only in terms of quality of fish (an essential element for electronic tagging), but also numbers for CT tagging. Overall, jigging accounted for a little over half (**Table 2**) of the total tagged fish on the trip. Obviously, in terms of physical effort and time, jigging is a much less efficient way to capture fish. But with the right team that enjoys jigging,

can handle the physical demands, possesses the technical expertise, and is properly equipped, jigging can be a highly effective way to accomplish objectives.

Meanwhile, trolling and dangling seem to have taken a smaller role in CP cruises in an apparent shift of fish away from TAO anchored moorings and onto dFADs, which are omnipresent. The highest number of tags in a dFAD dangling school, 575, pales in comparison to the nearly 1800 tags by a single TAO school, seen in CP cruises before this shift. However, the geographic distribution of tags seems to have benefited from this change (**Figure 3**). Anecdotally, it appears that no discernible pattern was noticeable for when trolling (in particular, danglers) was effective, with variables such as meteorological conditions, size of fish, apparent school composition, oceanographic conditions, and time of day factored into consideration. Patience and consistency seemed to be the most important factor in ensuring larger releases on the dFADs with trolling and dangling gear.

Larger (80cm+) bigeye played an important role in the catch composition of the trip and at times dominated what was available to be caught at certain dFADs. On four occasions, these larger bigeye came up on the dangler and troll gear and broke mono, straightened hooks, broke dangler poles, and ripped themselves off of hooks. Although completely inefficient (but a spectacular show), some CTs were still deployed in these schools. Access to this larger size class of fish was particularly useful for archival and sonic tagging, allowing for deployment of ATs in mostly 87cm+ fish. This is a significant difference from past CP cruises where the large bigeye were the exception to the rule. Pending recaptures, this could elucidate some movement and behavior of this size class of fish and their affinity (or not) to dFADs during a life stage where they are largely hidden from the main fisheries of the region.

The spatial distribution of releases during CP-14 is novel as compared to past CP cruises which relied on TAO moorings. This is an exciting and new development in tagging analysis, and of course depends on recaptures in the coming months and hopefully years. Depending on how useful this strategy was, future CP cruises may be more dedicated to this approach rather than looking for the “big jag” which seems to be rare now.

F/V Gutsy Lady 4 proved again during this cruise to be the perfect platform for this type of experiment. Its long range, stability, ample space on the working deck and comfortable accommodations are making a combination hard to compete in this class of commercial fishing vessel. The skills of the captain and his crews are of course one of the main components that made this tuna tagging project a success.

Lessons Learned and Recommendations

1. A new Oceanbox or perhaps a more modern communication system should be considered. The firmware/software issues at the beginning of the trip were alarming. If this were to happen while at sea, it would have been disastrous. During CP-14, the system went down for no apparent reason, and then miraculously became functional again. Perhaps a satellite issue but for 5 hours there was no connection.
2. Future cruises must be prepared for larger fish. This was the first CP trip that on many occasions, the school was almost pure large-sized B and Y. Typically, we get some fish in this size class but nothing like CP-14. Luckily, the team was able to fabricate gear for jigging that was appropriate for the task at hand. Additionally, electric reels should be considered for

future cruises where a large jigging effort will be made to relieve the fishermen and make fight times shorter, especially on smaller fish.

3. Attempting drifting arrays around dFADs must be done in low current situations, preferably around 0.5kts. Regardless of conditions, the vessel should be prepared to tend lines as needed, to keep the array as close to it's original shape as possible.
4. SrCL injections in archivally tagged fish should be reconsidered or the applicator modified to avoid continual prodding of these valuable fish? This protocol was eliminated after the first 14 archival tags after we realized the issues with the protocol.
5. A dedicated berth for a media/outreach person could produce high quality results and not burden the scientific staff with this seemingly endless demand.

Appendix 1. Gutsy Lady 4 characteristics

Name of Vessel	GUTSY LADY 4
Owner of Vessel	Gutsy Lady 4 LLC
Port of Registration	Honolulu, Hawaii
Vessel Type	Fishing vessel
Flag	USA (US)
Hull Type/year built	Steel / 2001
WCPFC registration	1120347
IMO	8970469
MMSI	367571490
Length (LOA)	26.15m /
Beam	7.92m
Draft	4.5m
Tons Gross	170
Engines Make and Model	2x Cummins KTA 19 (600hp)
Call Sign	WDG 7854

Address of company owner	**Game Over LLC** **350 Ward Avenue, Ste 106-315** **Honolulu, HI 96814, USA** **Tel: +1 808 217 4539**

APPENDIX 2-revisions to live biopsy protocol

The best live biopsy protocol given available tools and fishing situations during CP14 was employed at cradle 1 (starboard aft) during jigging sessions, and joined the responsibilities initially divided between genetics runner and supervisor. The use of block markers was discarded prior to the switch from cradle two, and was not revived since tagger and genetic sampler voice recorders always concurred. A special table with non-skid cover was set up near the archival cradle for genetics equipment. The revised elements of the protocol are described here.

Personnel:

-Tagger (Marion)

-Genetic sampler (Giulia)

Responsibilities:

-Tagger:

1) Tag fish as normal
2) When the genetic sampler is present, there is only one fish in the cradle, and at least 50% of fish have been tagged without biopsy, tagger will step aside prior to releasing a fish and allow access to the genetic sampler
3) Record genetic sample collection on voice recorder
4) Release fish and continue tagging as normal

-Genetic sampler:

1) Open sterile packaging of single use biopsy tool (only touching packaging) and place open package in a holder so punch handle is available
2) Wash gloved hands in diluted bleach for 10 seconds
3) Pick up biopsy punch tool and single-use wipe without touching other surfaces and move to waiting position near tagging cradle 1
4) When the tagger indicates it is okay, step in, wipe biopsy sampling site briefly with single-use wipe, remove plastic cover from biopsy punch tool, and take tissue sample

5) Note last empty tagging block number while leaving the cradle and record on own voice recorder once away from the cradle. Occasionally also note corresponding genetic vial number. Useful to also comment if tissue sample size is insufficient, potentially contaminated (such as by fish splashing), etc.

Appendix 3. Specifics about daily activity, location and deployed tags.

Date	Area	Activity	Conventional Tags			Archival Tags			Acoustic Tags			MiniPAT		Total
			BET	SKJ	YFT	BET	SKJ	YFT	BET	SKJ	YFT	BUM	OCS	
15-Aug-2020	Kewalo Basin	Leave port 12:00												**0**
16-Aug-2020	U.S.	Steaming-gear prep												**0**
17-Aug-2020	U.S.	Steaming-gear prep												**0**
18-Aug-2020	Int'l Waters-5	Fishing-dFAD	50	9	163			2						**224**
19-Aug-2020	Int'l Waters-5	Fishing-dFAD	5		9									**14**
20-Aug-2020	Int'l Waters-5	Fishing-dFAD	93	6	64							1		**164**
21-Aug-2020	Int'l Waters-5	Fishing-dFAD	2	1	6			1						**10**
22-Aug-2020	Int'l Waters-5	Fishing-dFAD	101	5	30	2		1					2	**141**
23-Aug-2020	Int'l Waters-5	Fishing-dFAD	104	13	76	1		3						**197**
24-Aug-2020	Int'l Waters-5	Fishing-dFAD	58	8	39	1								**106**
25-Aug-2020	Int'l Waters-5	Fishing-TAO 5N 155W	97	9	39	1						1		**147**
26-Aug-2020	Kiribati-Line Islands	Fishing-dFAD	28	8	16	4		3						**59**
27-Aug-2020	Kiribati-Line Islands	Fishing-dFAD	18		10	1		2						**31**
28-Aug-2020	Kiribati-Line Islands	Fishing-dFAD	4	28	30									**62**
29-Aug-2020	Kiribati-Line Islands	Fishing-dFAD	293	30	348			1						**672**
30-Aug-2020	Kiribati-Line Islands	Fishing-dFAD	181	45	79			1						**306**
31-Aug-2020	Kiribati-Line Islands	Fishing-dFAD	34	3	44			2						**83**
01-Sep-2020	Kiribati-Line Islands	Fishing-dFAD	36	2	23			3						**64**
02-Sep-2020	Int'l Waters-5	Fishing-dFAD	174	3	51	8								**236**
03-Sep-2020	Int'l Waters-5	Fishing-dFAD	115	3	25	5							1	**149**
04-Sep-2020	Int'l Waters-5	Fishing-dFAD	178	5	5	1							1	**190**
05-Sep-2020	Int'l Waters-5	Fishing-dFAD	87	2	61	3								**153**
06-Sep-2020	Int'l Waters-5	Fishing-dFAD	53	4	32									**89**
07-Sep-2020	Int'l Waters-5	Fishing-TAO 5N 170W			4									**4**
08-Sep-2020	Int'l Waters-4	Fishing-dFAD	59	1	53	5								**118**
09-Sep-2020	Int'l Waters-5	Fishing-dFAD	93	2	54	1							1	**151**
10-Sep-2020	Int'l Waters-5	Fishing-dFAD and TAO 2N 170W	35	3	42									**80**
11-Sep-2020	Int'l Waters-5	Fishing-dFAD	143	2	26	4		1						**176**
12-Sep-2020	Int'l Waters-5	Fishing-dFAD	139	2	30									**171**
13-Sep-2020	Kiribati-PIPA	Fishing-dFAD and TAO 2S 170W	43	2	34									**79**
14-Sep-2020	Kiribati-PIPA	Fishing-dFAD	143	3	26	4								**176**
15-Sep-2020	Kiribati-PIPA	Fishing-dFAD	89	1	24									**114**
16-Sep-2020	Kiribati-PIPA	Fishing-dFAD and TAO 2S 170W	82	1	14									**97**
17-Sep-2020	Kiribati-PIPA	Fishing-dFAD	141		4	3			2					**150**
18-Sep-2020	Kiribati-PIPA	Fishing-dFAD	99		7				30					**136**
19-Sep-2020	Kiribati-PIPA	Drifting/acoustic												**0**
20-Sep-2020	Kiribati-PIPA	Fishing-TAO EQ 170W												**0**
21-Sep-2020	Int'l Waters-4	Fishing-dFAD and TAO 2N 170W	65	12	41									**118**
22-Sep-2020	Int'l Waters-4	Fishing-dFAD	55		17									**72**
23-Sep-2020	Int'l Waters-5	Fishing-dFAD	112	5	26									**143**
24-Sep-2020	Int'l Waters-5	Fishing-dFAD	612	37	49									**698**
25-Sep-2020	Int'l Waters-5	Fishing-dFAD	188	28	11									**227**
26-Sep-2020	Int'l Waters-5	Steaming												**0**
27-Sep-2020	Int'l Waters-5	Fishing-dFAD	35		9									**44**
28-Sep-2020	Int'l Waters-5	Fishing-dFAD	474	35	130									**639**
29-Sep-2020	Int'l Waters-5	Steaming-gear storage												**0**
30-Sep-2020	Int'l Waters-5	Steaming-gear storage										1		**1**
01-Oct-2020	U.S.	Steaming												**0**
02-Oct-2020	Kewalo Basin	In port												**0**
Totals			**4318**	**318**	**1751**	**44**	**-**	**20**	**32**	**-**	**-**	**3**	**5**	**6395**