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Project 35: Bigeye biology, and Project 35b: WCPFC Tuna Tissue Bank

WCPFC-SC12-2016/RP-P35-01

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EXECUTIVE SUMMARY

Key Points for SC12 – Project 35

1. Analysis of bigeye tuna otolith and gonad samples are underway to provide estimates of annual age and maturity status for the next stock assessment.
2. Project 35 will be completed at the end of 2016 and **regular analyses of specimens for future stock assessments should be budgeted for and aligned with the stock assessment schedule.**

Key Points for SC12 – Project 35b

3. The tuna tissue bank has had its first three formal third party applications for access to samples.
4. Procedures for granting access to the WCPFC tuna tissue bank by third parties have been tested and as a result **amended procedures should be discussed at SC12** (including the approval process for access to more detailed data via the web-tool for those designing research studies).
5. The annual cost of supporting the tuna tissue bank now that it is established is USD80,000. **The SC12 needs to decide if it wishes to place an indicative annual budget of USD80,000 continuing in 2019 (2017 and 2018 are already in the indicative budget).** This comprises USD55,000 for tuna tissue bank coordination and training for samplers and USD25,000 for sampling fees and freight.
6. Observer training and refresher courses continue. Over 5% of active senior observers contributing to the WCPFC Regional Observer Programme have been trained in biological sampling methods and procedures. WCPFC Regional Observer Programme training standards and training materials for biological sampling have been prepared and updated.
7. The web-based tool for WCPFC CCMs and external parties to query the WCPFC tuna tissue bank continues to be improved. The web database is currently accessed by over 4000 unique users from all over the world (up from 1550 at 30/06/2015).
8. **The deposits to the tuna tissue bank over the period 01 July 2015 through 30 June 2016 include an additional 1,713 specimens of which over 93% are from the five core species.**
9. Australia has provided access to their quarantine and sample storage infrastructure through CSIRO. To date this has been an in kind contribution to the operation of the tuna tissue bank however the longer term capability of CSIRO to provide this service as an in kind contribution is unknown.
10. The tuna tissue bank continues to be accessed by the Science Services Provider for WCPFC and a broad range of other organisations.

1. BACKGROUND

The Western and Central Pacific Fisheries Commission (WCPFC) has identified that information gaps in key biological parameters are reducing the reliability of current assessments and management measures for several large pelagic fish stocks in the Western and Central Pacific Ocean (WCPO). Recent analyses have demonstrated important spatial and temporal differences in the age, growth and reproductive biology's of tunas and billfishes which exert considerable influence on the estimation of fisheries reference points. To reduce these uncertainties they have prioritised the work programme of its scientific committee to undertake stock-wide studies on the age, growth and reproductive biology's of tunas and billfishes (Nicol et al. 2015).

Project 35 has been implemented over the last seven years (Nicol et al. 2011, 2014, 2015). It is designed to address the scientific committee's requirements for improved knowledge on bigeye tuna age, growth and reproductive biology. WCPFC has provided funding to collect 2500 otoliths and 300 gonads across the WCPO to estimate spatial variation in growth and reproductive biology. The European Union provided further funding in 2014 to extend this collection to other tuna and billfish species for the purposes of establishing a WCPFC tissue bank that would allow the WCPFC to have immediate access to biological material to answer stock biology and provenance questions. The project successfully met the sampling targets set through to mid-2015 (Nicol et al. 2015).

SC11 recommended that funding be continued to maintain Project 35: Bigeye Biology and WCPFC Tuna Tissue Bank, with particular emphasis on WCPO bigeye, yellowfin, and skipjack tunas (Anon 2015a). SC11 also recommended that the Commission adopt the "WCPFC Tissue Bank Access Protocols" developed within Project 35 and modified by ISG-2 at SC11 (Anon 2015a). Subsequently the Commission endorsed both recommendations (Anon 2015b).

In 2016 WCPFC has funded two projects to implement these recommendations. Those projects are Project 35 – Bigeye biology, and Project 35b – Tuna Tissue Bank. This report provides an update on progress for these two projects, and identifies key recommendations for ongoing work.

2. PROJECT 35 – BIGEYE BIOLOGY

Project 35 has been implemented over the last seven years. It is designed to address the scientific committee's requirements for improved knowledge on bigeye tuna age, growth and reproductive biology. SC10 recommended that Project 35 commence laboratory analyses of bigeye samples to provide updated estimates of bigeye tuna age and growth, and reproductive biology, for stock assessment purposes. This project runs to 31 December 2016.

2.1 CONTEXT

In 2008, the Western and Central Pacific Fisheries Commission (WCPFC) endorsed a "Comprehensive Research Plan on Pacific-wide Bigeye Growth and Reproductive Biology" and supported a pilot project to determine the sampling requirements for implementing this study, Project 35 (Anon 2008). In 2009, a work plan for the pilot project was finalised and biological samples were subsequently collected through a regional sampling program. In 2011, the pilot project was completed in collaboration with CSIRO. Preliminary estimates of bigeye age and maturity were obtained for the bigeye stock assessment regions 3 and 4 and the sensitivity of the bigeye stock assessment to these estimates was evaluated (Nicol et al. 2011). After reviewing Project 35, the WCPFC supported the continuation of the sampling and analysis program to gain a better understanding of bigeye age and maturity. In late 2014, the WCPFC endorsed the 'analysis phase' of Project 35 (Anon 2014). Specialised analysis of otoliths and ovaries is required to estimate length- and maturity-at-age of bigeye tuna for this project.

2.2 PROGRESS IN 2015-16

In 2016 CSIRO will provide estimates of annual age and maturity status of bigeye tuna from otoliths and ovaries collected under WCPFC Project 35. The age and maturity data, and associated estimates,

obtained through the project will include a focus on characterizing spatial and temporal variation in the growth of bigeye tuna. SPC and CSIRO will jointly participate in the analyses of the age and maturity data to generate growth curves and maturity ogives.

The project will be conducted in accordance with the methodology described in the pilot study (Nicol et al, 2011) presented to the WCPFC Scientific Committee in 2011. The specific analyses underway include:

- Age estimates for 1100 bigeye tuna from across the stock assessment areas, with most otoliths from the main fisheries. The age estimates will be based on readings of transversely sectioned otoliths (1000 with annual counts, 100 with daily counts) with otolith selection based on length stratification designed for estimation of age-length keys and spatial representativeness, and
- Maturity and reproductive assessments of 200 bigeye tuna based on readings of histologically sectioned ovaries.

The outputs of the analyses will be available for input into the 2017 bigeye tuna stock assessment. A final report on the project will be provided to WCPFC by 31 December 2016.

2.3 FUTURE WORK

This project will be finalised in late 2016. Consideration should be given to adopting a longer term plan of work to ensure age and maturity data to generate growth curves and maturity ogives, with focus on characterizing spatial and temporal variation in growth, are available for the key tuna stocks (following the agreed schedule for tuna stock assessment (Anon 2015a).

3. PROJECT 35B – WCPFC TUNA TISSUE BANK

The WCPFC Tuna Tissue Bank (TTB) has been established over several years and its ongoing operation is now funded by WCPFC through Project 35B. The objective of the project is to maintain the WCPFC TTB with particular emphasis on WCPO bigeye, yellowfin, albacore and skipjack tunas, and swordfish, and, to facilitate transmission of samples to specified researchers with due cognizance of the WCPFC TTB Access Protocols. SPC as the Scientific Services Provider is tasked to maintain and develop the WCPFC TTB and through the biological sampling programme expand the inventory of samples held. This project runs to 31 December 2018 with funding for 2017 and 2018 subject to the decisions of WCPFC 13 and WCPFC 14 respectively.

3.1 CONTEXT

WCPFC has established a TTB so that national and international fisheries research institutes can access the collections to undertake the necessary analyses to estimate spatial and temporal explicit age, growth and reproductive parameters, and genetics for stock structure for use in stock assessments. In a broader ecosystem context the collections will also be used for trophic and system studies including diet analyses, stable isotopes, mercury and other biochemical elements for trophic structure and movements and taxonomic studies. Previous projects have seen a system of observer training, training of trainers, sample kit distribution, observer sampling at-sea and port-sampler sampling in port, sample transfer and sample curation established so that researchers can access an online database (Biological Database System – BioDaSys) of the WCPFC TTB. Procedures for granting access to the WCPFC TTB by third parties have been established and implemented.

3.2 PROGRESS IN 2015-16

3.2.1 Infrastructure

The TTB has long-term storage facilities at SPC Headquarters in Noumea, New Caledonia and at CSIRO, Brisbane, Australia. The storage in Noumea has been expanded by over 25% this year to cope with demand. These facilities are currently being provided in kind to the project by both organisations.

Numerous short-term/staging storage facilities in the key ports of the WCPO have been established, with changes in 2015-16 identified (see Table 1). Note that strategic investment in a super-cold storage facility will be required in the next few years to ensure the longevity and relevance of the WCPFC TTB.

A central feature of the repository infrastructure is a relational database that catalogues the samples (BioDaSys – see section 3.2.7). Specific information includes sample number; all tissues that were collected from that individual; the condition of these samples; species and its measurements; all information on where each sample comes from and how and when it was collected; who collected the sample; the location(s) where it is currently stored, how it was transported and who transported it to its current location. Meta-data about the fishing/sampling trip that the sample came from is also included. A third aspect of the database catalogues the analysis of the samples. This includes a description of the laboratory analyses, WCPFC project number and the primary information derived from these analyses (e.g. sample weights, analyses performed and resulting estimates (e.g. age, reproductive status, chemical composition, etc.) and who undertook the analyses and their contact details. Subject to the approval of the data dissemination protocols by the Scientific Committee of the WCPFC it is expected all data will be available to institutions or organizations responsible for providing scientific advice in fisheries through the web-accessible component of the database.

Table 1. Locations and storage capacity for the WCPFC Tuna Tissue Bank.

Port	Country	Freezer Capacity	Comment
Noro	Solomon Islands	15 m ³ Blast freezer (-30°C)	Soltuna Cannery
Honiara	Solomon Islands	0.7 m ³ (-18°C)	Min. Fisheries and Marine Resources
Port Moresby	Papua New Guinea	0.7 m ³ (-18°C)	National Fisheries Authority
Kavieng	Papua New Guinea	0.7 m ³ (-18°C)	National Fisheries College
Rabaul	Papua New Guinea	0.7 m ³ (-18°C)	National Fisheries Authority
Lae	Papua New Guinea	0.7 m ³ (-18°C)	National Fisheries Authority
Madang	Papua New Guinea	0.7 m ³ (-18°C)	National Fisheries Authority
Wewak	Papua New Guinea	0.7 m ³ (-18°C)	National Fisheries Authority
Koror	Palau	0.1 m ³ (-18°C)	Natural Resources, Environment, Tourism
General Santos	Philippines	0.21 m ³ (-18°C) 15 m ³ Blast Freezer (-30°C)	Bureau of Fisheries and Aquatic Resources Well Delight Network Corporation
Kaohsiung	Chinese Taipei	0.7 m ³ (-18°C)	Sun Yat Sen University
Yaizu	Japan	15 m ³ (-18°C)	National Research Institute of Far Seas Fisheries, Shimizu
Pohnpei	Federated States of Micronesia	0.7 m ³ (-18°C)	Min. Resources and Development
Majuro	Marshall Islands	0.7 m ³ (-18°C) 15 m ³ Blast Freezer (-30°C) 15 m ³ Blast Freezer (-30°C)	Marshall Islands Marine Resources Authority Marshall Islands Fishing Venture Pan Pacific Foods cold storage
Honolulu	USA	10 m ³ (-18°C)	NOAA
Aiwo	Nauru	0.15 m ³ (-18°C)	Fisheries and Marine Resources Authority
Tarawa	Kiribati	15 m ³ Blast Freezer (-30°C)	Kiribati Fish Limited
Papaete	Polynesie francais	0.7 m ³ (-18°C)	Resources marine et minières
Pago Pago	American Samoa	0.5 m ³ (-18°C)	Min. Marine and Wildlife Resources
Apia	Samoa	0.5 m ³ (-18°C)	Min. Agriculture and Fisheries
Suva	Fiji	0.7 m ³ (-18°C)	Min. Fisheries and Forests
Port Villa	Vanuatu	0.2 m ³ (-18°C)	Min. Agriculture, Livestock, Forestry, Fisheries Biosecurity
Noumea	Nouvelle Calédonie	6.4 m ³ (-18°C)	SPC
Brisbane	Australia	20 m ³ Blast Freezer (-30°C)	CSIRO

3.2.2 Sample collection

To simplify the sampling numbering system as much as possible the WCPFC ROP Observers are issued with biological sampling kits that include sample tags that are already numbered (see Figure 1). The database tracks the distribution of kits and sample tags allowing the coordinators of the repository to

ascertain the status of sampling supplies allocated to each ROP Observer and to ensure that regional observer offices have sufficient stock to replenish observer supplies.

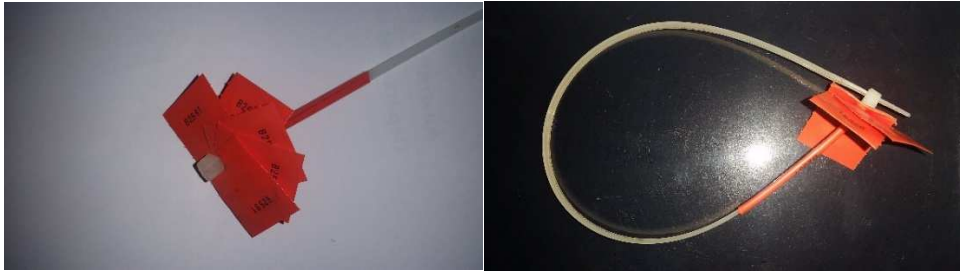


Figure 1. Photos of the cable tie tag that is issued to observers with unique numbers on them.

Biological sampling kits contain data sheets, pencils, knives, saws, cutters, cable tags, sample jars and bags, and instructions have been updated (see Figure 2 and Appendix I for *Sampling Instruction Sheets*). In 2015-16 instructions now also include swordfish head and anal ray sampling, blood sampling, and otolith extraction quality codes, as well as precise instruction for coordination at port. Gonad sampling and fixation instructions have also been developed for port sampling (see Appendix II for *Gonad sampling protocol*).



Figure 2. Examples of the equipment and supplied provided to observers in the biological sampling kits.

3.2.3 Tracking samples

The sample database (BioDaSys – see section 3.2.7) now also tracks sampling trips undertaken by observers, port sampling events, quality of the sampling, as well as payment of samplers allowing the sampling coordinators to follow vessels on which sampling is undertaken (Figure 3). This allows enhanced coordination of the reception of the samples, as well as the debriefing of the observer upon arrival. These changes have improved the ability to monitor the quality of the sampling undertaken and to coordinate payment for samples. In particular, the database allows validation of the sample collection position using VMS and logsheet tracks which increase the data quality control of the repository.

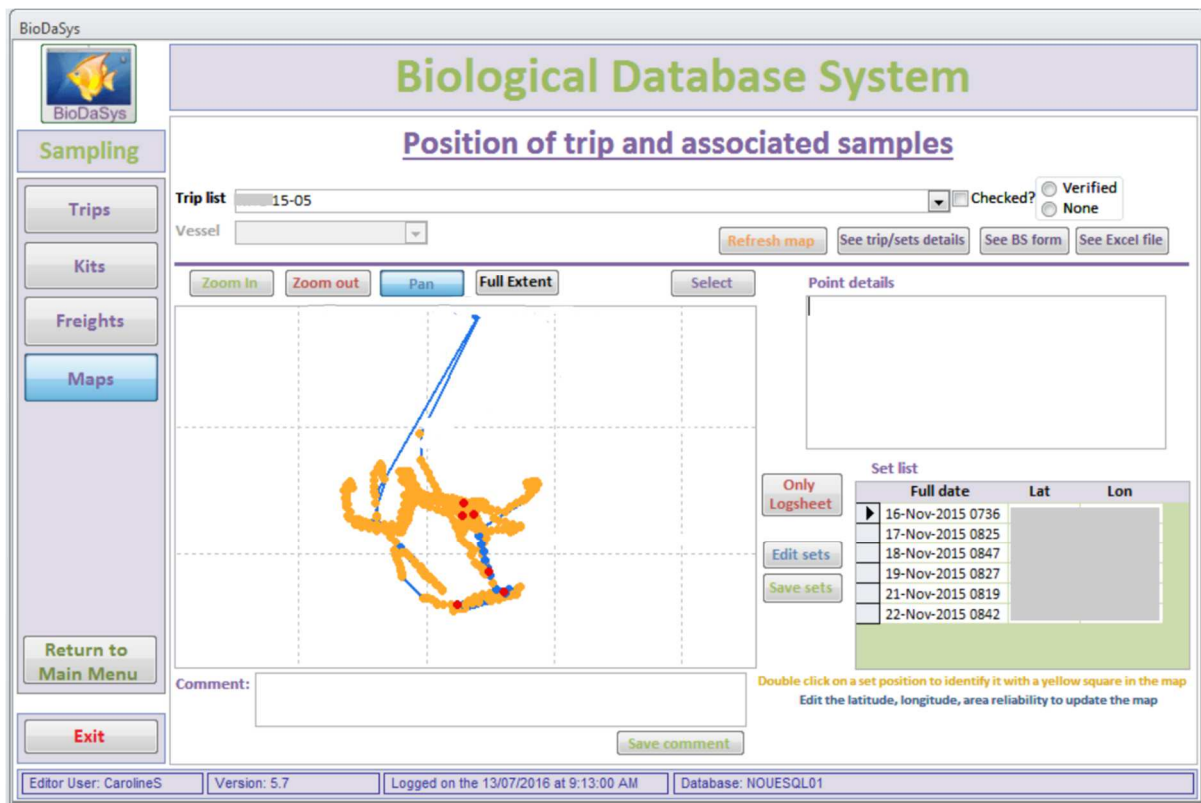


Figure 3. Example of trip information in BioDaSys as used by sampling coordinators to monitor and improve sample collection and data quality.

3.2.4 Observer Training Standards

Standards for training of observers in biological sampling have been prepared and accepted into the PIRFO training standards (see Appendix III for *PIRFO biological sampling competency standard*). An observer manual has been created and updated to the current sampling requirement (see Appendix IV for *Biological sampling manual for observers and port samplers*). A training manual has also been prepared (see Appendix V for *Observer training modules for biological sampling*) and is currently under revision to include new training procedures.

3.2.5 Observers and Observer Trainers

Senior observers that remain active in the WCPFC ROP and within National Programs continue to be identified and provided with training in biological sampling, including fish hard part extraction, tissue sampling, gonad sampling and data recording. Training has also included sample handling and transportation. An additional forty observers were trained in 2015-16 bringing the total trained to 459. This provides a sufficient number of observers for the collection of samples over the spatial domain of the WCPO and ensures that observers can be deployed on the appropriate vessel trips to implement a variety sampling strategies to meet WCPFC-SC requirements. Table 2 provides a summary of the changes this year in observer training by nationality. Table 3 provides a summary of the number of observer trainers who can now deliver biological sampling training by nationality. Refresher courses will be undertaken periodically for trainers.

Table 2. Summary of observers trained in biological sampling by nationality. Changes from 2014-15 indicate by ~~strikethrough~~.

Country	No. of OBS	Country	No. of OBS
Cook Islands	4 3	Papua New Guinea	79 69
Fiji	31	Palau	12
Federated States of Micronesia	64 48	Solomon Islands	63 52
Kiribati	47	Chinese Taipei	33
Marshall Islands	39	Tonga	13
Nauru	9	Tuvalu	10 9
Nouvelle Caledonie	2 1	Vanuatu	25
Polynesie francais	5	Samoa	23

Table 3. Summary of observer trainers trained to deliver biological sampling training by nationality.

Country	No. of trainers
Federated States of Micronesia	2
Kiribati	1
Marshall Islands	2
Nauru	1
Papua New Guinea	2
Solomon	2

3.2.6 TTB Sample Collections

Samples continue to be collected by national “at sea” and “port” observers across the WCPO. Observers collect to a strategy that minimizes the number of samples per set and maximizes sampling across sets and trips to create the greatest temporal spatial coverage possible. Opportunistic sampling on scientific cruises has also been undertaken.

In 2015-16 an additional 1,758 samples were deposited in the TTB (Table 4a). This comprised 692 yellowfin, 239 skipjack, 525 bigeye, 135 albacore, 10 swordfish and 17 striped marlin. The provisional total SPC Marine Specimen Bank incorporating the WCPFC TTB sample holdings to 30 June 2016 include 21,337 individual specimens (Table 4b). The tables below summarise the tissue samples per species. These data do not include samples awaiting cataloguing. Note the numbers of samples is greater than the number of fish as multiple samples are often available for the same fish (e.g. muscle tissue from different positions on the body, gonad samples in different storages). The quantity and details of such samples have not yet been verified due to the extended length of some observer trips, or the requirement to complete consecutive trips and the biological sampling information having not yet been submitted by the observer.

The distribution of samples by tissue type provided in Nicol et al. (2015) is now available via BioDaSys (see Section 3.2.7) and accordingly are no longer plotted in this paper. The rate of sampling of various species in key areas is monitored, and to the extent possible, observer tasking is directed to ensure spatial, species and temporal spread across the WCPO.

To recognise the effort involved in biological sampling and those who put in the effort, sampling appreciation certificates were distributed in late 2015 to encourage and acknowledge the work of the samplers across the WCPO (see Appendix VI *Certificates of appreciation*). It is intended to continue this recognition in future.

Table 4a. Summary of 2015-16 additions to the WCPFC Tuna Tissue Bank.

Species	Hard-parts			Reproduction	Multi-purpose				Diet
	Curated	Otoliths	Spines	Gonads	Blood	Muscle	Liver	Fin	Stomach
Bigeye	525	481	91	698	51	524	144		135
Yellowfin	692	634	253	830	106	708	348		310
Skipjack	239	214	209	233	24	237	235		236
Albacore	135	126	116	137	7	134	122		123
Swordfish	10	5	2	3	7	10	6	2	5
Striped Marlin	17		1	7	9	17	10		4
Wahoo	4			1		4	4		4
Mahi Mahi	37			3	16	37	22		22
Rainbow runner	9			1		9	9		9
Other#	45		6	12	36	45	31		7

Table 4b. Total holdings in the SPC Marine Specimen Bank incorporating the WCPFC TTB.

Species	Hard-parts			Reproduction	Multi-purpose				Diet
	Curated	Otoliths	Spines	Gonads	Blood	Muscle	Liver	Fin	Stomach
Bigeye	4109	2674	393	3125	71	2658	1368	0	635
Yellowfin	7775	3701	972	4199	167	5195	3824	0	1568
Skipjack	5314	1072	699	1055	104	3755	3704	0	1189
Albacore	4037	2321	773	2287	14	1153	1054	0	237
Swordfish	105	7	9	20	7	66	80	5	27
Striped marlin	99	0	1	7	1	44	41	0	4
Wahoo	333	28	0	6	0	216	217	0	8
Mahi Mahi	345	0	0	2	16	209	188	0	27
Rainbow runner	337	0	0	1	0	212	214	0	21
Others#	6384	3757	9	3202	23	852	2851	78	63

#includes lancetfishes, kawakawa, blue marlin, frigate and bullet tuna, moonfish, black marlin, escolar, spearfish, barracudas, mackerel scad, triggerfishes, blue shark, pelagic stingray, manta ray, silky shark, sailfish, Spanish mackerel, oilfish, short-finned and long-finned mako sharks, snake mackerel, pomfrets, trevallies, blue chub, oceanic white-tip shark, filefishes, batfishes, fangtooth, devil ray, sandbar shark, sergeant major, tiger shark, alfonsinos, amberjack, anchovies, bigeye thresher shark, bronze whaler shark, bull shark, unicornfish, crocodile shark, flying gurnards, gemfish, hammerhead sharks, reef sharks and squids.

3.2.7 Web Accessibility

A web-based tool has been implemented to allow WCPFC members to track the collection of samples (via BioDaSys). It includes interactive maps where the user can obtain information on the number, type, species and length classes of samples collected from particular EEZ and high seas areas (see Figure 4). An on-line query system is also included to allow more detailed information on each sample to be viewed (e.g. date and location of sample and types of samples taken from the individual, sample quality; see Figure 4). The web tool is currently available at: www.spc.int/tagging/webtagging/BioDaSys/. The on-line query tool has been accessed by over 4000 unique users from all over the world (Figure 5). The vast majority since February 2015. Since last year, 2270 users have accessed it.

The web interface will be progressively updated in the following months to follow the refactoring of the backend system named BioDaSys (Biological Database System) which has been completely redeveloped in order to support the WCPFC TTB. Initially a system to manage biological sampling in the Pacific Ocean (trips, sampled fish, samples and associated analysis), BioDaSys is now a full-featured infrastructure to allow for example to verify and validate data provided by samplers (see section 3.2.3), to track freights, samples movements and pending analysis. A range of developments continue including developing clear protocols on the way specimens are stored and related information in the database, the physical size of a specimen on arrival (e.g. volume or mass for a muscle sample) and changes to the sample size as a result of any use through approved access to the TTB.

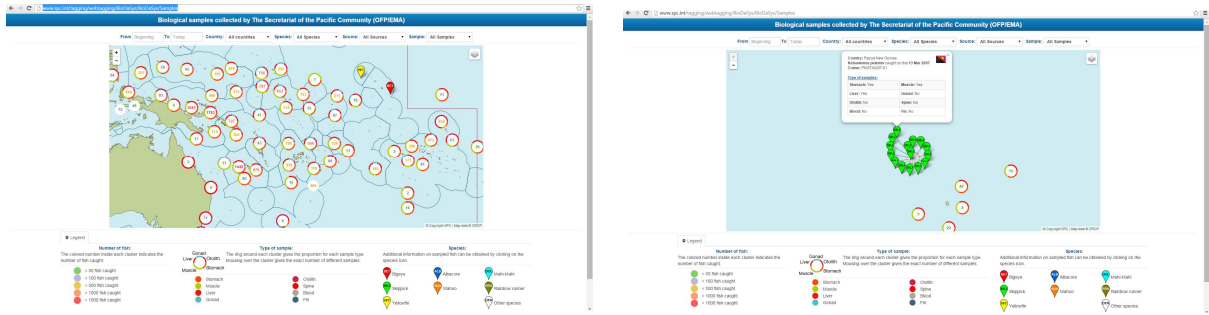


Figure 4. Web query tool for the WCPFC Tuna Tissue Bank.

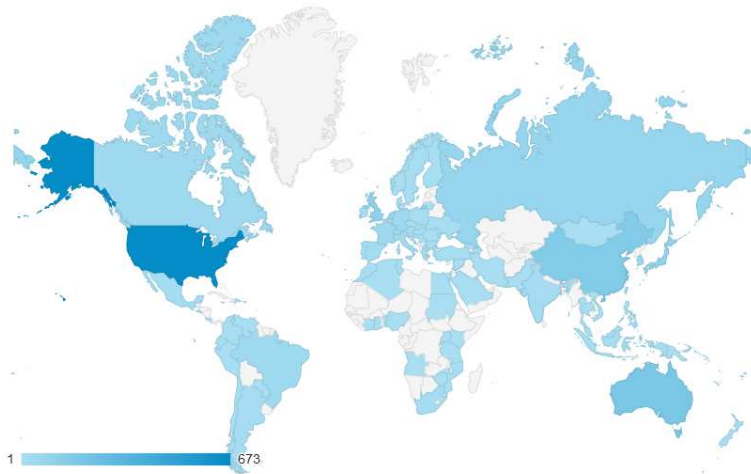


Figure 5. Global distribution of BioDaSYS web tool unique users by country (noting that for 27% of users, no country of origin is available).

At present the more detailed information – as is generally needed to design research studies – is not available without an approved login. At present there is no official process for obtaining an approved login. Accordingly, SPC needs to prepare a data extract subject to the WCPFC data access rules and provide that extract to potential researchers. To facilitate better use of the TTB and the BioDaSys web-tool it is proposed that those researchers wishing to access more detailed information be able to apply for a login directly from the Scientific Services Provider. Only those data fields necessary to design research would be accessible to authenticated users. Any specific request for samples would still require approval via the access protocol (see Section 3.2.8).

3.2.8 Tuna Tissue Bank Access

Making samples available to third party organisations for analyses maybe an option that the Scientific Committee pursues to fast track certain analyses. A protocol for accessing the TTB for subsequent laboratory and data analyses by third party organisations was adopted by the WCPFC-SC in 2015 (Anon 2015a).

Apart from pre-approved WCPFC projects (e.g. CSIRO work on bigeye tuna under Project 35), there have been three requests to withdrawals from the TTB in 2015-16. All three requests have been sent to the WCPFC Research Sub-Committee for approval, with a result pending. Table 5 outlines the projects that have previously and/or are currently accessing the TTB for WCPFC work, including the three recent requests.

Table 5. Projects that have previously or currently access the WCPFC Tuna Bank (new for 2015-16).

Project Description	Samples Used	Technique	Organisation	WCPFC-SC Project No
Age and Growth				
Bigeye Growth Curves	Otolith	Ageing	SPC CSIRO Sun Yat-Sen University	35
Albacore Growth Curves	Otolith	Ageing	SPC CSIRO	39
Swordfish Growth Curves	Otolith/Spines	Ageing	CSIRO	71
Reproductive Biology				
Bigeye Maturity Ogives	Gonads	Histology	SPC CSIRO	35
Albacore Maturity Ogives	Gonads	Histology	SPC CSIRO	39
Albacore Reproductive Biology	Gonads	Histology	SPC CSIRO	39
Trophic dynamics				
Ecosystem Effects of Fishing	Stomach Muscle Survey	Diet Analyses DNA metabarcoding Taxonomy Fatty Acid	SPC University Canberra Curtin University CSIRO	37, 46
FAD impacts on trophic dynamics	Muscle Liver	Isotope	SPC University Southampton	37
Ecosystem and species Biogeography	Stomach	Diet Analyses	SPC University of Tokyo	TBP
PNG Long-term Climate Monitoring	Stomach e-DNA	Diet Analyses DNA metabarcoding	SPC University Canberra Curtin University	TBP
SEAPODYM	Stomach e-DNA	Diet Analyses DNA metabarcoding	SPC University Canberra Curtin University	62
<u>Global scale analysis of tropical food web dynamics to understand climate impact on top predators (swordfish and the four main tunas)</u>	<u>Muscle</u>	<u>Stable isotope analyses, fatty acid analyses</u>	<u>IRD SPC CSIRO</u>	<u>62</u>
Movement				
South Pacific Albacore	Otolith	Trace Element	SPC	38
Spatial Variations in concentrations of metal contaminants in food webs of the South Pacific Ocean	Muscle Blood	Isotopes & Mercury	IRD/SPC	TBP
Stock Provenance				
Indonesia-west Pacific tropical tuna stock structure	Fin	DNA - Microsatellite	CSIRO	TBP
Global tropical tuna stock structure	Fin	DNA – NGS	University Bologna	TBP
Albacore	Muscle	DNA - mitochondrial	AZTI	TBP
<u>Black marlin</u>	<u>Muscle, liver</u>	<u>DNA - SNP</u>	<u>University of Queensland</u>	<u>TBP TBP</u>
<u>WCPO tuna stock structure and movement for albacore, skipjack, yellowfin and bigeye</u>	<u>Muscle</u>	<u>DNA - SNP</u>	<u>University of the South Pacific, CSIRO</u>	
Food Safety				
Spatial Variations in concentrations of metal contaminants in food webs of the South Pacific Ocean	Muscle Blood	Mercury Accumulation	IRD/SPC	TBP
Marine plastic pollution and seafood safety	Stomach	Composition	CSIRO	TBP

*TBP = To Be Provided

3.2.8 Tuna Tissue Bank Access Protocol

A TTB Access Protocol was approved in 2015 (Anon 2015b) and is available on the TTB web page. Several practical issues with the protocol have been identified through 2015-16 as a result of implementing the protocol. The issues identified include:

- i. Access to meta-data to plan research proposals – this is identified and discussed in Section 3.2.7. The list of sample features and data fields to be released need to be agreed. It is noted that with respect to the location of the samples (which are linked to fishing operations in the case of observer sampling), latitude and longitude are critical for scientific research, but also potentially sensitive. It is recommended that sample locations be released at 1 degree resolution for research design purposes. A multi-level login is proposed, with regular authorised users in one category, authorised users for a specific individual project (time-bound) and general access. The information available at each level would be based on need.
- ii. Timelines for approval – there is currently no timeline identified for the review of proposals. It would seem prudent to identify a timeline to allow researchers to better plan activities, and to encourage timely applications from potential researchers.
- iii. Details to be included in applications – one area the data held in BioDaSys is being expanded is the physical size of the specimen (e.g. volume, or mass). This allows consideration of multiple use of the same specimen. Accordingly, applicants should specify the physical size of the sample required so that decisions about priority for destructive testing can be better informed. Further applications need to explicitly identify if the specimen will be destroyed as part of the analytical process.

As a result it is recommended that the protocol be amended to address these issues, with suggested wording given in Table 6, with ~~deletions~~ and additions. Note that some of the suggested changes are simply editorial in nature.

Some additional gaps identified in the implementation of the WCPFC TTB are a standard form of agreement for those withdrawing specimens (to cover specimen and data use, reporting obligations, recovery of costs and intellectual property) and protocols on managing the longevity of specimens in the bank. A standard form of agreement for those withdrawing specimens is currently under development. Protocols on managing the longevity of specimens in the bank will be developed in advance of SC13 for discussion at that meeting.

3.3 FUTURE WORK

This project is intended to be ongoing. Given the success of the TTB to date, consideration should be given to incorporating the budget into the 2019 indicative budget. The following recommendations arise from this report on the TTB in 2015-16. Note that most should be completed within the existing proposed budget. Where additional resources would be required, they are identified.

In addition to maintaining and operating the TTB, in 2016-17 proposed enhancement work includes:

- a. Further investment in training standards (see Section 3.2.4) and in observer and observer trainer training (see Section 3.2.5) to establish biological sampling as a ROP observer core duty ensuring that the repository continues to develop – note that this requires support from the TTC, but does not require additional resourcing at this time
- b. Development of protocols for standard TTB extraction approaches and having such protocols stored on BioDaSys (e.g. for otoliths for sectioning)
- c. Additional resources are required to assist with coordinating the transfer of samples from the observer to storage facilities – this includes resourcing access to Australian quarantine and sample storage infrastructure through CSIRO
- d. Development of protocols on managing the longevity of specimens in the bank, and
- e. Designing and seeking funding for strategic investment in a super-cold storage facility, required to ensure the longevity and relevance of the WCPFC TTB.

Table 6. Suggested amendments to the WCPFC Tuna Bank access protocol with ~~deletions~~ and additions. Note that clauses not amended from the adopted protocol are not included in the table.

Background

1. The WCPFC has established a tuna tissue bank of biological samples collected from pelagic species in the WCPO for the purposes of ~~life-history~~ studies to advance fisheries management in the WCPO. The bank contains otoliths, dorsal spines, gonads, liver, muscle, stomachs and blood from tuna, billfish and other pelagic species.
2. The purpose of this document is to specify the rules for third-party scientific researchers to access and use these samples for the purpose of scientific study. Research projects contracted by the WCPFC requiring access to the tissue bank will have access protocols agreed in the procurement process.

Rules and Procedures

- X. (new) Applications to access the web-data tool for meta-data describing samples in the planning stages of a project should be sought from the WCPFC Scientific Services Provider. Note that such a log-in will only allow project appropriate access and will be time limited.
3. Applications to access samples from the tissue bank should be addressed to the Executive Director, WCPFC Secretariat, and must include:
 - a. ~~Applications should be addressed to the Executive Director, WCPFC Secretariat~~
 - b. Project Name and Objectives
 - c. WCPFC Scientific Committee Project Number or recommendation if these exist
 - d. Specification of the samples to be withdrawn from the bank (number, type, species, size of sample/proportion of available sample to be used, any location/sex/date limits, etc.)
 - e. The methods for processing and analyses (in particular whether the method will destroy part or all of the sample, and what sample record will be retained, e.g. sectioned otolith slides)
6. Where the analyses involves the preparation of secondary products such as sectioned otoliths and histological slides these products are to be provided to the WCPFC Tissue Bank at the completion of the study for curation, future comparative reference and study.
8. The selection and approval of projects will be determined by the WCPFC Research Sub-Committee. This committee may meet within the margins of WCPFC meetings or electronically. This sub-committee will prepare and submit a summary of their decision on each project proposal to the WCFPC Executive Director for final approval. Decisions should be taken within 30 days of the application being received. The project approval process will consider, inter alia, the following:
 - a. Preferential access to the tissue bank will be given to researchers or WCPFC CCM's who have contributed samples to the collection.
 - b. Preferential access to the tissue bank will be given to collaborative projects with priority to those where the collaboration includes the Science Services Provider for WCPFC and more than one several WCPFC CCMs.

4. ACKNOWLEDGEMENTS

For 2015-16 the TTB has been funded by WCPFC through Project 35b. Previously the development of the TTB has been generously supported by WCPFC through Project 35, the European Union through the European Maritime and Fisheries Fund, and Australia, IRD and SPC with in kind and direct funding. We are grateful for the provision of storage and sample distribution by the range of agencies identified in Table 1. A special thanks to the observers, observer trainers and observer managers across the region that make the TTB possible. Also special thanks to the staff of the agencies co-ordinating biological sampling across the region including: Marshall Islands Marine Resources Authority, Marshall Islands; Ministry of Fisheries and Forests, Fiji Islands; Department of Resources and Development, Federated States of Micronesia; National Fisheries Authority, Papua New Guinea; and Ministry of Natural Resources, Environment and Tourism, Palau. We are also very grateful to the support received from Luen Thai in Majuro, Kiribati Fish Limited (KFL) in Tarawa and Soltuna in Noro for access to fish and providing support to observer biological sampling.

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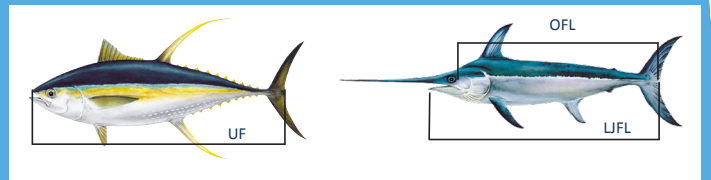
APPENDIX I

Sampling instruction sheets

BIOLOGICAL SAMPLING PAMPHLET

HOW TO MEASURE THE FISH

- Measure the fish: UF for tuna species, and both LJFL and OFL for swordfish. Note the OFL in the comments section of the form. If for any reason you cannot measure the LJFL, explain this in the comments section.
- For tuna species make sure that you only sample 5 fish for the size range concerned.



HOW TO USE THE CABLE TIE LABELS



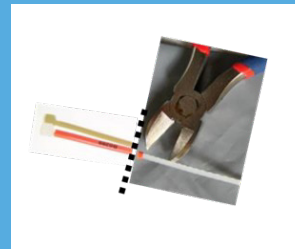
- Place the cable tie with 5 labels through the mouth of the fish.



- Once it is attached, gently pull on it to ensure it will hold. If the otoliths are extracted at port you should leave the cable tie on the fish.



- Remove the labels from the cable tie and place them inside the plastic bag with the biological samples.



- Cut the orange part (with the number) from the cable tie and place it with the otoliths inside the vial.



Each fish has 1 cable tie with several identically numbered labels. All the samples coming from one fish must have the same label number! Note this label number on the biological sampling form.

HOW TO SAMPLE BLOOD

- When the fish is killed, while it is bleeding place the vial under the blood dripping from the fish. Try to fill the whole vial (a minimum of 10 ml is required).
- Before closing the vial place a label between the vial and the lid – while screwing the lid the label will be secured by the pressure of the lid against the vial. Gently pull on the label to ensure it will hold. Store the vial in a freezer.



HOW TO EXTRACT THE OTOLITHS FROM TUNA

Use an appropriate method to extract the otoliths. The method will depend on the size of the fish and whether it is necessary to keep the fish in good condition for commercial purposes. When extracting the otolith be careful in positioning the tweezers to avoid losing the otolith inside the brain cavity.



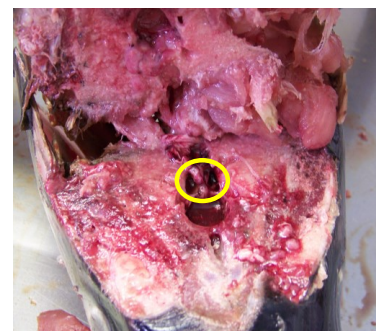
Tweezers in lateral position



If you are able to extract otoliths on board, try to extract the otoliths before collecting any other samples. For any fish, if you cannot remove at least 1 otolith do not continue to collect other biological samples for that fish. Stop the sampling, remove the cable tie label, and sample another fish.

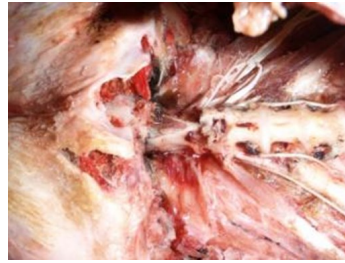
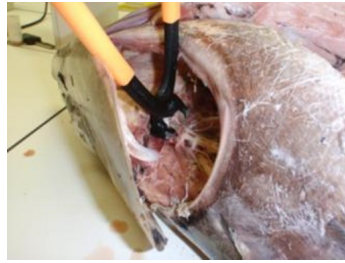
Remove the top of the head

- If necessary, remove the head of the fish from the rest of the body before removing the top. Stabilise the head on the floor.
- Cut straight down past the top of the eye. Hold the head towards you.
- Remove the brain with the back end of the tweezers.



Cut the otic capsule with nail removers and side cutters

- Remove the large lump of bone from the bone mass inside the gill opening with the nail remover to reveal a 'V' shape in the remaining bone mass.
- Use the side cutters to clear the remaining bone to expose the otolith cavities.



Drill cores under the gills

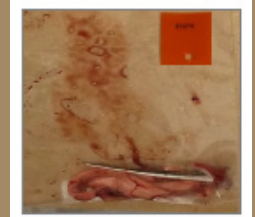
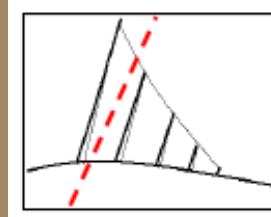
- Open the operculum to insert the drill, and press the drill against the bone lump at an angle of 45 degrees (towards the opposite eye).
- Drill both sides. Use the back of the tweezers to remove the bone from the saw hole.

- Remove the membrane around the otoliths, clean and dry the otoliths (the otolith should be completely white without any trace of blood). Place them in a vial with the cable label (do not add water or alcohol in the vial).
- Do not freeze them !



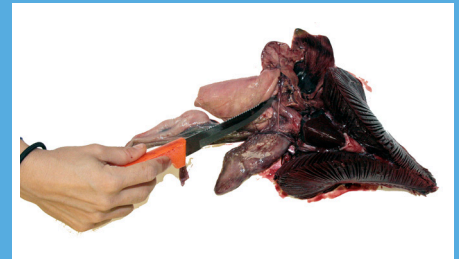
FIRST DORSAL SPINE SAMPLING

- Use a knife to cut the membrane between the first and second dorsal spines. Place one hand behind the first dorsal spine and push it forward towards the head of the fish. Grasp the first dorsal spine and swing it left to right a few times until the spine is unlocked from its base. Firmly pull on the first dorsal spine to remove it from its base.
- Place the spine in the bag with the gonads, making sure it lays flat to prevent it piercing the bag.



HOW TO COLLECT OTHER INTERNAL BIOLOGICAL SAMPLES

- When the fish is gilled and gutted, put aside the guts.
- Cut 4-5 cm of the liver – about the size of an average finger.
- Place the liver + 1 label inside a small plastic bag.
- Cut the stomach away from the digestive system.



- Cut the oesophagus as close as possible to the gills.

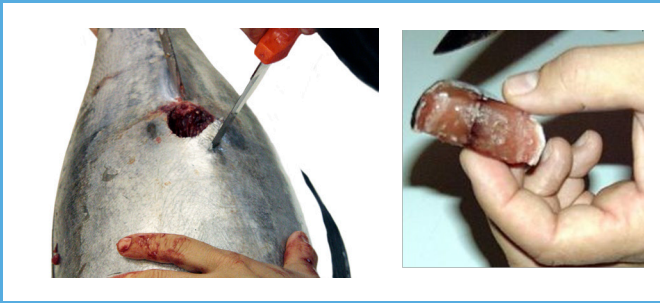


- Place the stomach + 1 label inside a large plastic bag.



➤ Cut a 4-5 cm sample of muscle from the back of the fish, or from near the anus (preferably from the back).

➤ Remove the skin from this sample and place the muscle +1 label inside a small plastic bag.



➤ Find the gonads of the fish – if it is not with the guts, it will be inside the belly of the fish towards the backbone.

➤ Carefully remove the gonads. Check inside the fish again to ensure that you have collected the entire gonad.

➤ Place the 2 gonads +1 label inside a plastic bag.



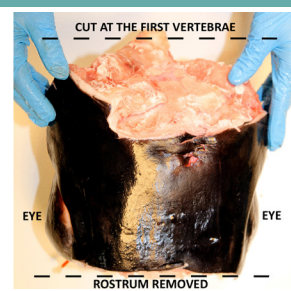
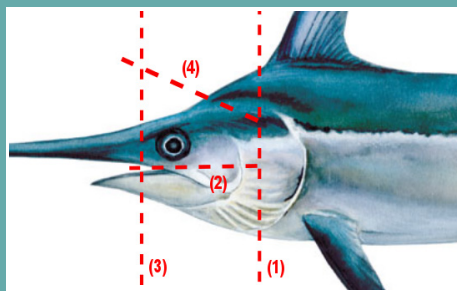
If the fish is not yet mature and you cannot identify the sex of the fish, you must still collect the gonads.



When you are sampling several fish, do not leave the samples under the sun. Place all samples immediately in an esky, a bucket of ice, or directly in the freezer.

HOW TO SAMPLE A SWORDFISH HEAD

➤ Remove the head from the rest of the body – the cut is done at the operculum section, leaving the first vertebrae with the rest of the body (1). Remove the lower jaw from the head (2). Cut the rostrum in front of the eye (3). Remove the upper part of the head (4).



➤ Remove the side of the head, including the eye. Repeat for the other side. These cuts aim to reduce the sample size before the packaging.

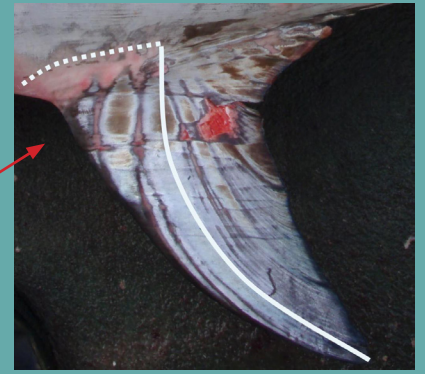
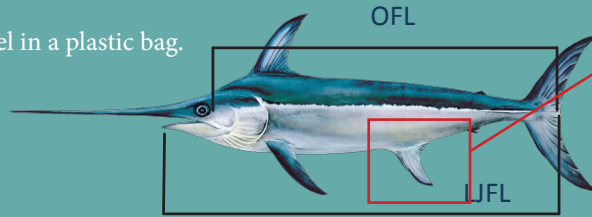
➤ Place the head section in a large plastic bag, together with the cable tie. Remove as much air as possible from the plastic bag.



Use the same label number for internal organs, fin rays and otoliths.

HOW TO COLLECT ANAL FIN RAYS FROM SWORDFISH

- Separate the second fin ray from the first and third ray. Slice through the fin with a knife (white line on the picture).
- Cut the skin around the fin ray and remove the base of the ray embedded in the flesh (dashed line on the picture).
- Place the second fin ray and a label in a plastic bag.
- If the second ray cannot be identified remove the entire anal fin.



WHAT TO DO AFTER SAMPLING A FISH?

- Fill in the biological sampling form. Note on the biological sampling form the details of the fish that has been sampled, the label number, and all the samples collected. You can use the spine column to note the fin ray sample from swordfish and/or the blood samples. If the Spine column has already been filled in, you can specify the blood sample in the Comments column.

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



- Place the bags on top of each other and roll up all of the samples coming from a single fish. Make sure the label is visible and placed on top of the bag, so the number can be read later on. If you have sampled blood you don't need to roll the blood sample with the other samples.



Once all sampling is completed, the samples need to be stored inside a freezer the entire trip. Ensure that the cook and/or the crew don't eat your samples!

- Use the Sampling Protocol page as a checklist for the fish that you have sampled. Mark a vertical bar in the appropriate box for each fish you have sampled so you can count how many fish you sampled from a specific size range during the trip. For example : III means 3 fish sampled, IIII means 4 fish sampled, IIII means 5 fish sampled.

Example of Sampling Protocol page:

Size range (cm)	30-40	40-50	50-60	60-70	70-80	80-90	90-100	100-110
Skipjack (SKJ)			###	III				
Bigeye (BET)				I	III			
Yellowfin (YFT)			I					
Albacore (ALB)								

WHAT TO DO BEFORE ARRIVAL TO PORT ?

- Before arriving at port, gather all of your frozen samples in a large plastic bag and label the bag with your ID number and the disembarking country name. Use a permanent pen to write the information on the large plastic bag.
- When the fishing vessel is 3/4 full, contact **Caroline Sanchez**: carolines@spc.int / +687 242227.
- Send her your approximate arrival date and port and she will coordinate collection of the samples between you and the local fisheries authority or the fishing company.
- You must ensure that the samples are stored on shore in a freezer.



Do not leave the samples in the fishing vessel unless you have no other choice, and if you do so immediately inform Caroline as well as the local fisheries authority.



Pacific Community
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Western and Central Pacific Fisheries Commission

APPENDIX II

Gonads sampling protocol

Gonads sampling protocol for histological analyses

If you have a scale and you collect the entire gonads, **weight both gonads** (you can still weight the gonads even if they are broken, place all the pieces on the scale). Note the weight of the gonads in the comment section of the BS form. There is no need to weight if you cannot collect the entire gonad.

Then, depending on the size of the gonads, there are 2 protocols:

- If gonads are larger than the cassette, cut a wide slice and put it in a container.
- If gonads are small, cut a 5mm slice and put it in a cassette (5mm is the height of the cassette).

Formalin is dangerous: handle carefully and don't breathe too close.

Sampling big gonads

As you can see on this picture, these gonads are larger than the cassette.



1- Cut a wide slice: around 5cm wide, the eggs need to hold together.



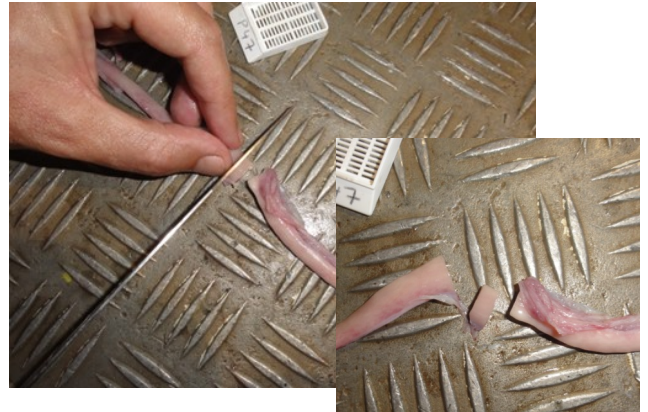
- 2- Put the slice in a 250ml container.
- 3- add 10% formalin till the formalin covers the slice (5cm higher than the slice).
- 4- Write the sample number, sampling date and vessel name on the container cap **with a permanent marker** (do not use a pencil).
- 5- Store the container(s) in a cool place, hidden from sunlight.
- 6- After 2 weeks, remove formalin from the vial and put some 70% ethanol instead. On the cap , write 70% ethanol instead of 10% formalin.

Sampling small gonads

1- Write the sample number on the cassette with a drawing pencil (the top of the cassette is removed by a light twisting).

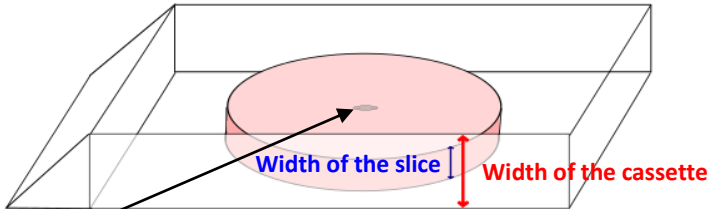


2- Cut a 5mm wide slice (5mm is the height of the cassette).



3- Lay the slice flat in the cassette. When closing the cassette, make sure the top of the cassette don't squeeze the slice. If it does, remove the slice and cut it thinner to fit inside the cassette. The width of the slice must be smaller than the width of the cassette.

4- Close the cassette and put it in the 10% formalin (you can put several cassettes in the same container).



Center of the gonad
(small hole) facing up



5- Write the sampling date and vessel name on the container cap **with a permanent marker** (do not use a pencil).

6- Store the container(s) in a cool place, hidden from sunlight.

7- After 2 weeks, remove formalin from the vial and put some 70% ethanol instead. On the cap , write 70% ethanol instead of 10% formalin.

DO NOT MIX SAMPLES COMING FROM SEVERAL BOATS IN THE SAME CONTAINER



For assistance, contact Caroline Sanchez: (carolines@spc.int) or
François Rounsard (francoisr@spc.int)



APPENDIX III
**PIRFO biological sampling
competency standards**

PIRFO Competency Standards

NEW PIRFO 3-6.05	Carry out biological catch sampling in accordance with a pre-determined sampling protocol
<p>Prerequisites:</p> <p>Descriptor</p> <p>This module requires candidates to understand the protocols of a biological sampling program, correctly identify anatomical parts of a fish, and participate in a pre-determined biological sampling program through accurately collecting and recording samples as required by the sampling protocol</p>	
Learning Outcome	Assessment Criteria
1. Demonstrate knowledge of established sampling programs employed in regional Pacific tuna fisheries	1.1 The main sampling programs used in regional tuna fisheries are outlined 1.2 The use of information obtained from sampling programs is summarised
2. Show awareness of fisheries observer roles and tasks in relation to regional sampling programs	2.1 Roles and tasks in sampling programs are summarised and include: <ul style="list-style-type: none"> • fish tag reporting and recording • stomach contents collection • otoliths and other hard part identification and removal • tissue and blood sampling 2.2 Demonstrate knowledge of the importance of compliance with sampling program protocols and record keeping standards
3. Awareness of fisheries observer roles in tagging programs	3.1 Fisheries Observer roles in relation to marine and fish species tagging programs are understood
4. Identify key internal organs and fish body parts commonly collected in sampling programs	4.1 Internal fish anatomy is correctly described 4.2 Key internal organs are identified and located 4.3 Methods for determining fish sex are described
5. Demonstrate practical biological fish sampling skills	5.1 Demonstrate safe use of the tools that are used to carry out biological sampling of fish 5.2 Selected organs or body parts are removed from fish samples, and stored and recorded in accordance with specified procedures

PIRFO Competency Standards

PIRFO 3 - 6.05

Evidence and Assessment Guide

Context and Method of assessment

Assessment is to be conducted at the workplace or in a simulated workplace environment.

The following assessment methods are suggested:

- practical exercises
- written or oral short answer testing
- observation of practical demonstration.

Resources for assessment may include:

- fish samples
- sampling protocols specified by the Regional Observer Program

Underpinning knowledge

Candidates are required to demonstrate general knowledge of sampling programs in place regionally in Pacific fisheries and the roles of fisheries observers in relation to these sampling programs.

Candidates need to be able to correctly identify the internal organs of fish species that are the target of these sampling programs

Learning Outcome	Evidence Guide
1. Demonstrate knowledge of established sampling programs employed in regional Pacific tuna fisheries	Candidates should be aware of biological sampling initiatives and the type of information collected in such programs. They should also be aware of regional tagging programs and the ways in which information from tagging programs is used
2. Show awareness of fisheries observer roles and tasks in relation to regional sampling programs	Candidates should be familiar with the roles of fisheries observers in relation to sampling programs. This includes: <ul style="list-style-type: none"> • the requirements for fish tag reporting and recording • sampling programs for stomach contents collection • otolith and other hard part identification and removal • tissue sampling. Candidates should also demonstrate an understanding of the importance of sampling program protocols and accurate record keeping.
3. Awareness of fisheries observer roles in tagging programs	Candidates should know how to record and report the landing of a tagged fish, bird or marine mammal.
4. Identify key internal organs and fish body part commonly collected in sampling programs	Candidates should be able to identify and locate the main internal organs and otoliths in selected fish species Candidates to know the established methods for determining sex in selected fish species
5. Demonstrate practical biological fish sampling skills	This element requires candidates to carry out practical dissection demonstration using correct techniques to cut fish, identify and remove selected organs or hard parts. Candidates should also demonstrate correct methodology for labeling and recording samples.

PIRFO Competency Standards

Practical skills

The essential skills a person needs to perform work to the required standard include:

- The ability to correctly select and dissect species and remove selected body parts
- The ability to record data and store samples in accordance with agreed protocols

Literacy skills used for:

- Interpretation of information about sampling programs
- Collection of data and information
- Accurate completion of forms

Numeracy skills used for:

- Recording data

Critical aspects of evidence

Assessment must confirm an ability to:

- Record the information required for a tagging or sampling program
- Correctly identify the sex of selected species of fish
- Dissect a fish to identify and remove key organs and hard parts

Assessment must confirm awareness of:

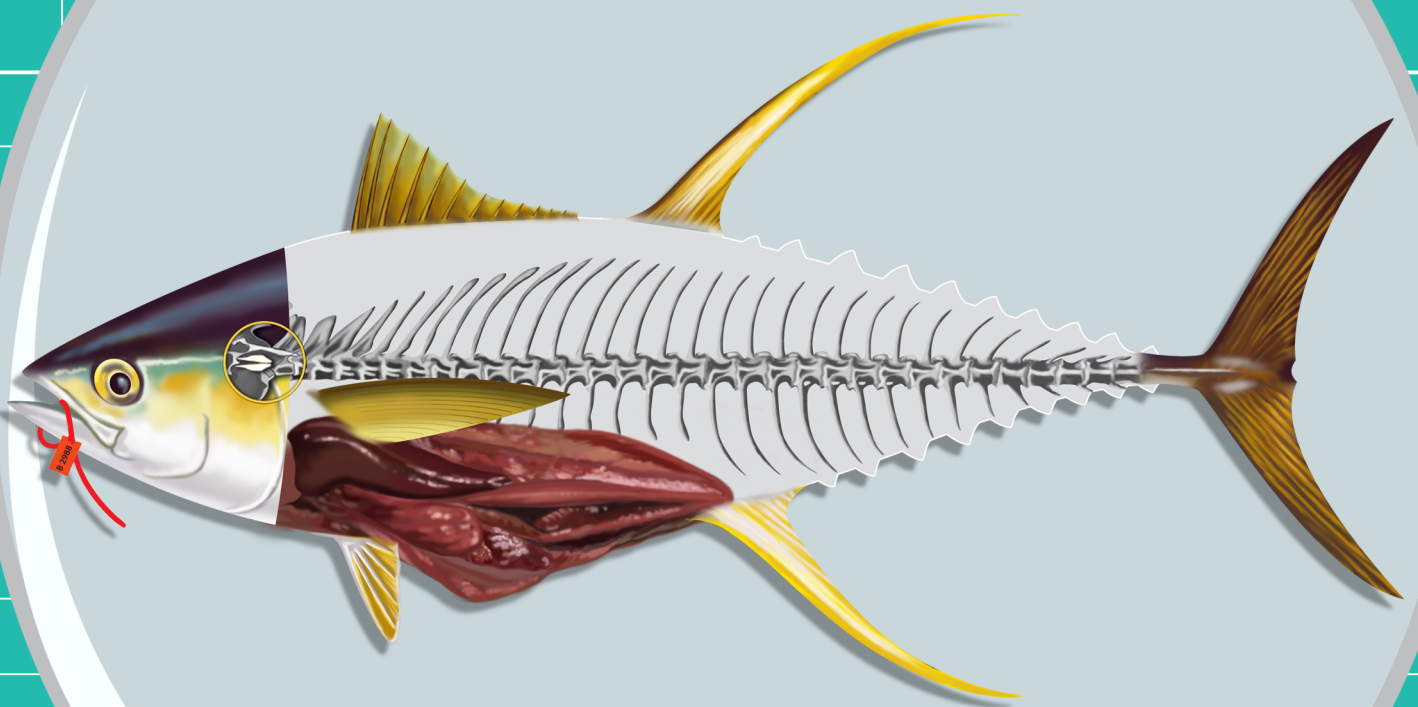
- The main sampling and tagging programs of Pacific fisheries
- How sampling and tagging information is used and why it is important
- Characteristics of key internal body parts of selected species

APPENDIX IV

Biological sampling manual for
observers and port samplers

Biological Sampling manual

Guide for observers / Port samplers



Pacific
Community
Communauté
du Pacifique

This manual is intended for observers and port samplers collecting stomach, muscle, liver, gonad, otolith and dorsal spine samples from yellowfin, bigeye, albacore and skipjack tunas and bycatch species in the Western and Central Pacific Ocean.

The Oceanic Fisheries Programme of the Pacific Community (SPC) aims to provide science-based information to member countries to assist them in making decisions regarding the conservation and sustainability of their tuna resources. Information obtained from sample analyses makes it possible to refine knowledge of tuna biology and ecology. Ultimately, multiple types of data are integrated to understand trophic relationships between tuna and their environment, as well as to produce species- and country-specific stock assessments that help member countries and territories manage their fisheries in a sustainable way.

1. The scientific projects

Several projects involving biological sampling are being undertaken by the Fisheries Ecosystem Monitoring and Analysis Section at SPC.

Ecosystem studies aim to improve understanding of the ecosystem that supports tuna fisheries by studying the diet of tunas and bycatch species. The basis of this work consists of sampling tuna stomachs, muscles and livers.

Reproductive and growth biology studies aim to improve the understanding of population dynamics for these species by providing estimates of growth rates, fecundity, and age and size at maturity. To achieve this, otoliths, gonads and dorsal spines are collected.

Mercury studies aim to understand patterns and accumulation of methyl mercury in top predators, to track tuna migration through mercury levels and reveal potential health issues. To achieve this, observers are asked to collect blood and muscles.

Genomic studies aim to describe the tuna population genetic across the Pacific region in order to assist management policy and to provide practical markers for fishery independent verification of catch provenance. Genetic analyses can be undertaken from samples such as muscles, gonads and blood.

The collection of samples started in 2001, allowing the creation of a Pacific Marine Specimen Bank, from which samples can be withdrawn for specific scientific projects. If there is a specific collection requirement, SPC or your observer coordinator will instruct you as to what species of fish to sample, what sizes, how many, and what kinds of samples are needed from each species.

2. Before going onboard

During observer placement the fishing company and the captain must be informed that you will be doing biological sampling onboard, as well as which type of sampling you will undertake.

If it is necessary to freeze the samples, ensure that you can store your samples in a freezer and that there is a specific area set aside for them where they won't be damaged.

3. Biological samples

Seven types of biological samples can be collected:

1. stomachs
2. muscles
3. livers
4. gonads
5. otoliths
6. dorsal spines
7. blood

This sampling is mostly done onboard by observers embarking on purse seiners and longliners. It can also be done in port during port sampling. Here we detail the step-by-step methodology for sampling.

Do not sample a fish whose size cannot be measured, for example a fish that has been damaged by a shark.

Unless you are directed otherwise by your coordinator, you can sample all sizes of fish. If many fish are being caught and are therefore available for sampling try to sample fish across a wide range of sizes. Follow sampling instructions provided to you for specific projects.

If you are sampling stomachs do not choose a fish where the stomach has been turned inside out and has popped out of the mouth. The stomachs are used to study diet, and if the prey are regurgitated the stomach cannot be analysed.



Figure 1: Albacore tuna with stomach popped out. Do not sample fish in this condition.

4. Labelling the fish and samples

The cable ties indicate which fish have been sampled, to identify between fish on the deck, and at port when the otoliths are removed.

The cable tie ID can have several tear-off labels, one of which is placed in each sampling bag. This number is the ID number of the fish.

Samples from each individual fish have a unique identifying number written on a label. Write down the number on the biological sampling form for each fish before beginning sampling. When you place samples in the bags, please ensure that the number on the labels can be read. Place the label in top left corner of the sampling bags. Use a drop of water to make it stick to the bag. This is important for listing and checking the samples as well as when samples are sorted for laboratory analysis.



Figure 2: Cable tie ID number. All the samples from an individual fish must have the same number.



Figure 3: Place the cable tie through the mouth of the fish. Ensure it is not going to fall off by gently pulling on it.

5. Collecting the biological samples

Muscle sampling

Depending on the species, muscle samples can be taken from around the anus, on the back of the fish, or from other parts (such as the wing for the ray).

If samples are from the back ensure that the fish is either rejected or that the captain agrees to its sampling. Generally, for tuna, the back will be sampled only if it is a rejected fish or on purse seiners.



General sampling:

1. Cut the muscle sample around the anus where it has already been cut for gutting the fish (4–5 cm² – about size of an average finger).
2. **Remove the skin** from the muscle sample.
3. Place the muscle sample in a medium-sized bag with a label that has the number facing outwards.
4. If the muscle is taken from the anal area, write 'A' on the biological sampling form. If it is taken from the back, write 'B', and if it is taken from any other part of the fish, specify the code on your form. For these areas (other than the anus) you must ask permission from the captain to sample the fish.

Note: collecting sample muscle from the back is the primary choice of sampling. You should collect samples from another area only if it is not possible to sample from the back.



Figure 4: Sampling muscle (anal position) and placing it in a medium-sized bag.

Note:

.....

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Liver sampling

1. Cut 4–5 cm² of the liver – about the size of an average finger. Ensure you sample the liver (dark colour) and not the digestive system (lighter colour).
2. Put the liver sample inside a small plastic bag with a label.

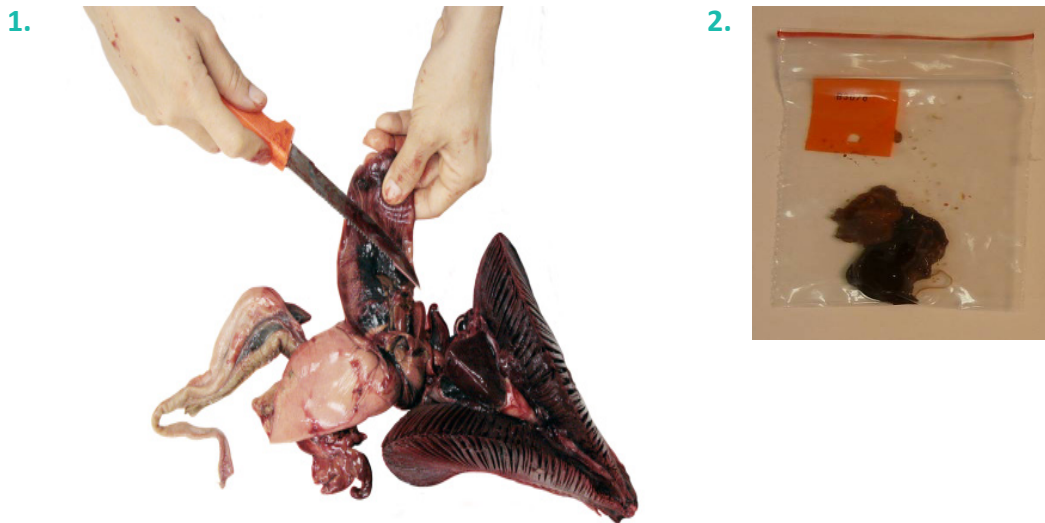


Figure 5: Sampling the liver and placing the sample in a small bag.

Stomach sampling

1. Cut the stomach away from the digestive system.
2. Cut the oesophagus as close as possible to the gills.
3. Remove the stomach.
4. Place the stomach and a label inside a large plastic bag.
5. Place the label on the top of the sampling bag; use a drop of water to make it hold.

If the stomach does not close properly you can use a small piece of rope to close the stomach at the site of the oesophagus cut. If you notice there are prey still in the oesophagus or that some have fallen out of the stomach, pick them up and place them in the sampling bag. Write a note in the comments section explaining this.

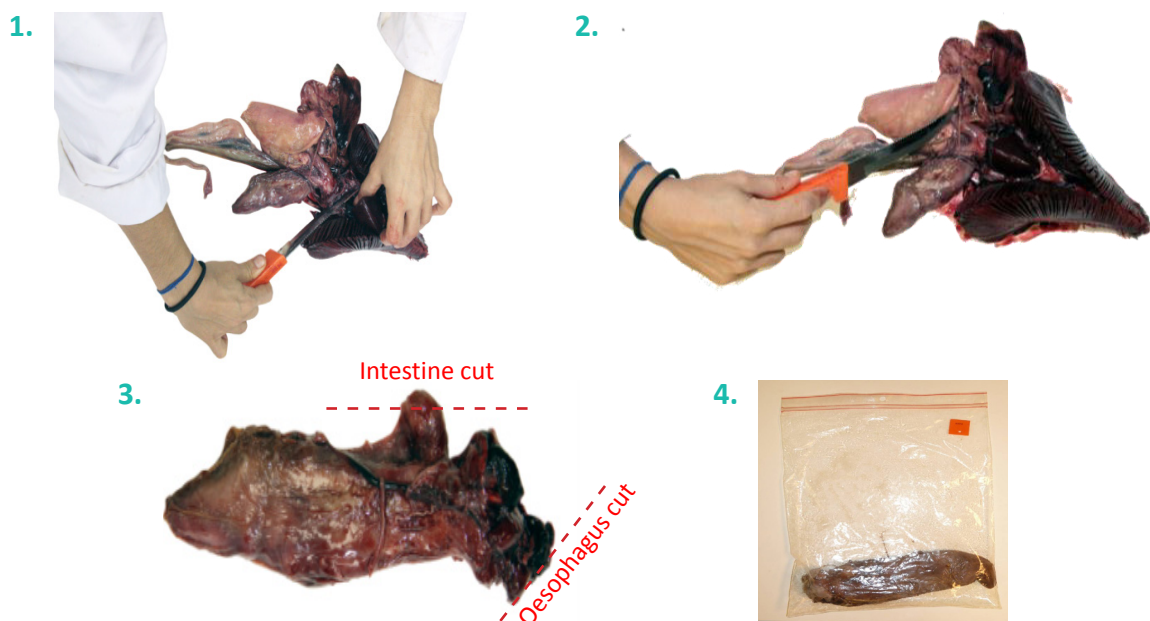


Figure 6: Stomach sampling (steps 1 to 4).

Otolith extraction

You can use several methods to extract otoliths, depending on the size of the fish and whether it is necessary to keep the fish in good condition for commercial purposes.

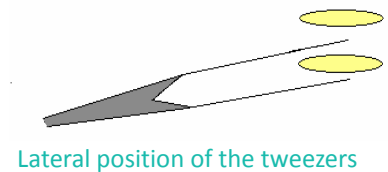
Destructive techniques:

1. Remove entire head (for very small fish) and leave aside (frozen) for someone else to sample (with a cable tie label through the mouth).
2. Remove the top of the head using a saw.

Non-destructive techniques:

3. Drill cores under the gills using the drill and hole saw (for big specimens).
4. Cut the otic capsule with nail removers and side cutters.

You will always need tweezers to remove the otoliths from the otic capsule. When the tweezers come into contact with the otoliths the sound is very different to that of bone. If you break the otoliths, keep all the pieces and place them together in the vial (see chapter 6 how to note this information on the biological sampling form)



When extracting the otolith be careful in positioning the tweezers to avoid losing the otolith inside the brain cavity.

Once you have removed the otoliths from the otic capsule remove the surrounding membrane, clean the otoliths (rinse and dry) and place them in a vial with the cable tie label (do not add water or alcohol in the vial). Ensure that there is no trace of blood before placing the otoliths inside the vial. **Do not freeze them.**

Removing the top of the head technique (a)

Depending on the size of the fish, you can either remove the entire head before cutting the top of the head, or you can keep the fish whole. Before extracting the otoliths, stabilise the head or secure the whole fish from rolling around by either using your legs to block the fish, or blocking the fish against any strong vertical wall found on the fishing vessel. In order to locate the otoliths cavity, place the head facing you. Remove the brain with the back end of the tweezers.

Cut the otic capsule using cutters (b)

After the gills have been removed locate the otic capsule where the backbone joins the head. Remove the large lump of bone from the bone mass inside the gill opening with the nail remover to reveal a 'V' shape in the remaining bone mass, then use the side cutters to clear the remaining bone to expose the otolith cavities.

Drill cores under the gills (c)

After the fish has been gilled and gutted, open the operculum to slide in the drill, and press the drill against the bone lump at an angle of 45 degrees (toward the opposite eye). Drill both sides, pull back the drill while it is still running, then stop it when the bone is fully extracted from the head. Use the back of the tweezers to remove the bone from the saw hole (ensure the drill is on safety lock). Locate the otoliths inside the bone.

Remove the entire head from the swordfish (d)

Remove the head from the rest of the body – the cut is done at the operculum section, leaving the first vertebrae with the rest of the body (1). Remove the lower jaw from the head (2). Cut the rostrum in front of the eye (3). Remove the upper part of the head (4). Remove the side of the head, including the eye. Repeat for the other side. These cuts aim to reduce the sample size before the packaging. Place the head section in a large plastic bag, together with the cable tie. Remove as much air as possible from the plastic bag.



Figure 8: Extraction methods for otolith sampling using a saw, cutters and a drill. You can cut the stem of the cable tie label and place it in the jar with the otoliths.

First dorsal spine sampling

1. Use a knife to cut the membrane between the first and second dorsal spines.
2. Place one hand behind the first dorsal spine and push it forwards, towards the head of the fish.
3. Grasp the first dorsal spine and swing it left to right a few times until the spine is unlocked from its base.
4. Firmly pull on the first dorsal spine to remove it from its base.
5. Place the spine in the bag with the gonads, ensuring it lays flat to prevent it from piercing the bag.

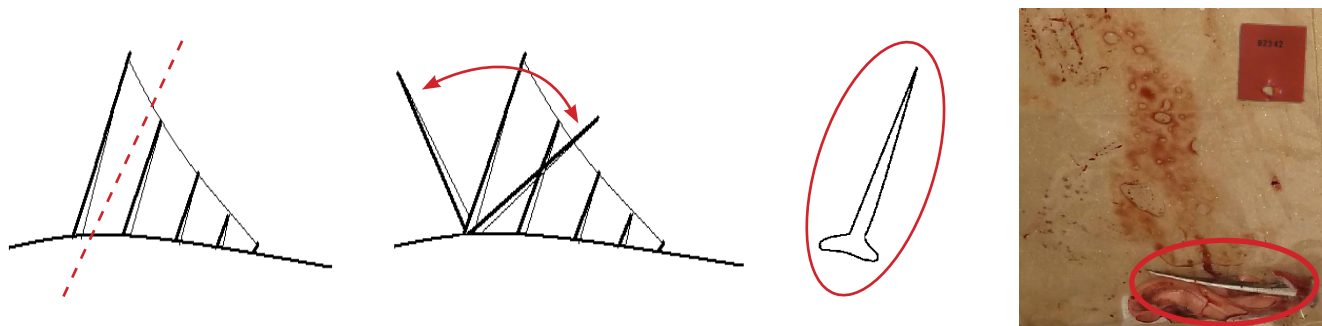
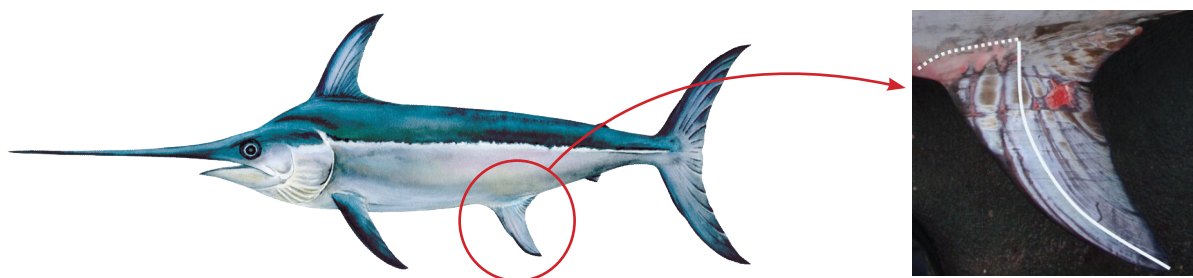


Figure 9: First dorsal spine sampling. Ensure the spine lays flat alongside the gonads.

Second anal fin ray sampling – Swordfish

The anal fin ray can be used in the same way as the otolith to identify the age of the fish. To separate the second fin ray from the first and third ray, slice through the second fin ray with a knife. Cut the skin around the fin ray and remove the base of the fin ray embedded in the flesh (see dashed line on the picture for cutting guide).



Blood sampling

After the fish is killed, while it is still bleeding place the vial under the blood dripping from the fish. Try to fill the whole vial (a minimum of 10 ml is required). Before closing the vial place a label between the vial and the lid – while screwing the lid the label will be secured by the pressure of the lid against the vial. Gently pull on the label to ensure it will hold. Store the vial in a freezer.

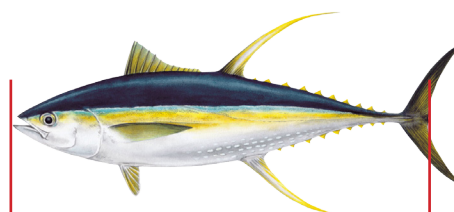


6. Recording data on the biological sampling form

Data recording is very important. We cannot use the results from analyses of the biological samples without good data. Ensure you record the date and time, the position of the set where the fish was sampled, and its size.

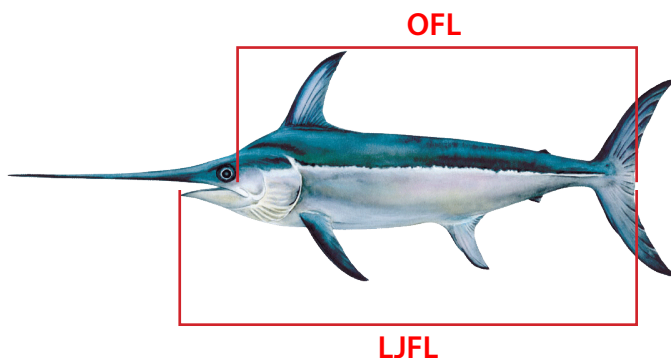
The information on the biological sampling form must be linked to the numbered labels, which will be placed in the sampling bags containing the samples. Therefore, do not forget to write the label number on the form.

For whole fish, take a UF code measurement, (upper jaw to the fork in the tail). Length measurements are rounded down to the nearest whole centimetre (e.g. 65.7 cm = 65 cm).



UF

For swordfish, take both LJFL (Lower Jaw to Fork Length) and OFL (Eye Fork Length). Note the OFL in the comments section of the form. If for any reason you cannot measure the LJFL, explain this in the comments section.



All information about biological samples is recorded on the **biological sampling form**. For purse seiners (PS), use this form only, and for longliners (LL), match the information on this form with the information on the LL-4 form.

The information includes: sampler name, observer trip number, name of vessel, start and end of trip dates, page numbers, start of set date and time, position, school association code (for PS only), ship's time (for LL only), condition of the fish on the deck (if it is alive or dead), label number (fish ID), species code, length and code, sex, the types of samples collected for each fish, muscle site and comments.

Use the 'comments' column to specify if you collected two otoliths, or only one otolith, if one of them is broken or both of them are broken. See below the code to describe the otolith extraction, write in the 'comments' column the appropriate code.

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

If you are undertaking a sampling not listed in the columns, but there is a 'Samples type' column that will not be used, change the title of the column by making a note next to it (see example below).

If all the 'Samples type' columns are used and you need an extra column to note a sample, use the 'Comments' columns and note the sample collected (see example below).

You can use the spine column to note the fin ray sample from swordfish and/or the blood samples. If the 'Spine' column has already been filled in, you can specify the blood sample in the 'Comments' column.

Use the 'General comments' columns to record any difficulties encountered during sampling (such as loss of samples, a broken item, if the captain did not allow sampling this fish, the crew ate the gonads, the stomach was popped out, etc.).

BIOLOGICAL SAMPLING FORM																	
REVISED at SPC - June 2016																	
SAMPLER NAME			OBS. TRIP ID NUMBER			VESSEL NAME			GEAR TYPE		START OF TRIP DATE		END OF TRIP DATE		PAGE OF		
Caroline SANCHEZ			CCS 1601			FUKUICHI MARU N=112			PS		16 / 02 / 15		16 / 03 / 16		01 / 05		
START OF SET DATE AND TIME	LATITUDE	LONGITUDE	PS-2 SCHOOL ASSOC	LL-4 SHIP'S TIME	CONDITION (A or D)	LABEL NUMBER	SPECIES CODE	LENGTH (cm)	SEX (M,F,U,I)	Y/N	STOMACH	LIVER	MUSCLE SITE (A,B,W,T)	SPINE	OTOLITHS	COMMENTS	
MM DD hh mm	(dd°mm.mmm') N S	(ddd°mm.mmm') E W															
02 18 06 30	04°23.150N	150°30.122E	3	-	D	B5241	BET	110	UF	F	Y	Y	Y	B	Y	Y	Oto 2G Blood sampled
02 18 06 30	04°23.150N	150°30.122E	3	-	A	B5242	SWD	196	LF	F	Y	Y	Y	B	N	Y	Head 189 OFL See comments
02 18 06 30	04°23.150N	150°30.122E	3	-	D	B5243	YFT	103	UF	M	Y	Y	Y	B	Y	Y	Oto 2G Gonads Broken
General comments: B5242 = Gonads throw away by crew while sampling.																	

Figure 10: Example of a biological sampling form showing additional samples listed in the 'Comments' column, status of the otoliths, and other relevant comments related to the sampling

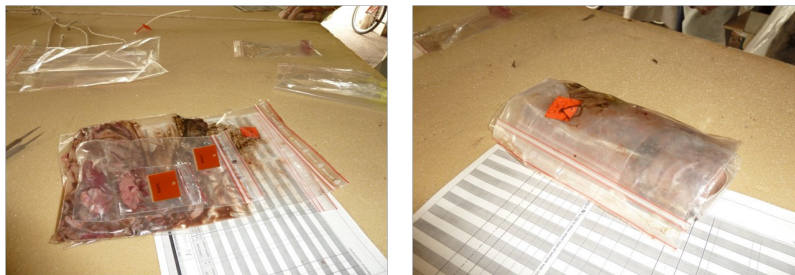
7. Packaging the samples

Place the bags on top of each other and **roll up all of the samples** coming from a single fish. If you use a rope to attach the samples together do not squeeze the samples with the rope.

Make sure the **label is visible** and placed on top of the bag, so the number can be read later on. If you have sampled blood you don't need to roll the blood sample with the other samples.

When you have collected the samples, **put them in the freezer as soon as possible**. For example, during sampling place the samples in the shade, if time allows and you have a lot of fish to sample. Once you have sampled 5 fishes, before continuing the sampling, place the samples in the freezer so they do not stay out in the heat too long. This is very important for stomach samples, as the digestion process continues after the stomach has been removed. If you cannot easily access the freezer and you have more than 5 fish to sample, you could place the samples in a bucket of ice.

Ensure all of your samples have a label and store them together in a large solid plastic bag. Use a permanent pen to write on the large bag your **trip ID number** and the **disembarking port name**.



Place the vials containing the **otoliths in a dry re-sealable bag** in a safe place. Don't freeze them. Be aware if you fly with the otoliths vials to seal the caps of the vials with some sticky tape or electric tape. This will ensure that the lid does not pop up with the pressure variation in the aeroplane.

Ensure when storing the samples onboard that the bag is either labelled or the crew (especially the cook) knows about it so the samples don't get thrown away or used for cooking.

8. Arrival at port

When the fishing vessel is 3/4 full, contact Caroline Sanchez: carolines@spc.int/+687 242227. Send her your approximate arrival date and port and she will coordinate collection of the samples between you and the local fisheries authority or the fishing company.

You must ensure that the samples are stored on shore in a freezer. If you disembark do not leave the samples on the fishing vessel unless you have no other choice, and if you do so immediately inform Caroline as well as the local fisheries authority.

If you are continuing your trip onboard the same vessel and there is no freezer or cold storage available onshore, the samples can remain onboard the vessel: inform Caroline as well as the local fisheries authority.

When you get back to port, hand over the samples and the data to the observer coordinator or the debriefer. During the pre-debriefing, let the debriefer know of any difficulties you may have had with the sampling procedures.

For further information or any questions regarding biological sampling, kits, protocols and shipment, contact Caroline Sanchez (carolines@spc.int) or Francois Roupsard (francoisr@spc.int).

Thank you very much for your valuable collaboration on these scientific projects, which will help to manage our oceanic fisheries.

Example of biological sampling – debriefing checklist

Observer name: _____ TRIP ID: _____ Vessel name: _____ Debriefing name: _____

Debriefing Date (dd/mm/yy): / /

Biological sampling Form (BS form):

- Trip details are noted and confirmed.
- No empty field. For all the samples, fields “N” or “Y” must be noted.
Note: If the muscle is collected, the site where the muscle was collected must be noted.
- Observer trip ID is written as followed: OBS ID code + year (YY) + nb. trip this year (00).
Note: If you have a FFA trip, note together the FFA trip as well as the general OBS trip ID
- If a sample is not collected or if a sample is missing, a comment should be noted.
Note: Use the general comments or individual comments. Depending on the scientific project, some samples are not required.
- Check if observer followed the sampling instructions.
Note: current project, sample no more than 5 fish / species/ size range / trip. Use of the protocol check list (table)
- Check the position “Start of set”
Note: remember that we need the position of start of set, not the accurate catch position.

Samples:

- Check the samples collected. What is recorded on the BS form match the samples collected?
Note: On the BS form, use a colour pen to circle the mistake and note the correct information.

-If for a sample it is noted “Y” but the sample is not collected, circle the “Y” and replace it by “N”.
-If for a sample it is noted “N” but the sample is collected, circle the “N” and replace it by “Y”.
-If a field is empty check the samples to find out if the sample has been collected or not and correct the information on the BS form.
- Check the labels. Each sample must be placed individually in a plastic bag with a label.
Note: gonads and dorsal spine can be placed in the same plastic bag.

-If a sample is labelled but not recorded on the BS form, Note a comment on the BS Form.
“Missing: Specify the label number, sample type”. For example: “Missing B24561, stomach and liver”.
-If a sample is not labelled. Note a comment on the BS Form: “Not Labelled: Specify the sample type”.
-If a sample is not labelled but rolled with other samples from the same fish, use a pencil and write the missing label number on a waterproof/water resistant paper and place the label with the sample.
- If several samples are not labelled. Check if you can identify from which fish the samples are coming from. Look at the sex of the fish and what type of gonads were sampled, look at the size of the fish.
-Same label is recorded 2 times on the BS form: circle the mistake. Look at the label number to find out which label is missing on the BS form.
- All samples coming from the same trip are gathered in a large plastic bag with a label (obs trip id, port of arrival)
- Additional sampling requirement for the trip followed by OBS (specific project based requirement) *Note:* _____

Debriefing comments: _____





Pacific
Community

Communauté
du Pacifique



Western and
Central Pacific
Fisheries
Commission

APPENDIX V

Observer training modules for biological sampling

**Training Guidelines for Delivering:
Biological sampling, Tag Recovery and Tag
Seeding Competency Units**



Pacific Island Regional Fisheries Observer



SECRETARIAT OF THE PACIFIC COMMUNITY
SECRÉTARIAT GÉNÉRAL DE LA COMMUNAUTÉ DU PACIFIQUE



The Biological Sampling, Tag Recovery and Tag Seeding are competency units part of the
Pacific Island Regional Fisheries Observer Certification and Training Standards

Secretariat of the Pacific Community

Oceanic Fisheries Programme

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<http://www.spc.int/oceanfish/en/ofpsection/fisheries-monitoring/observers>

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Introduction

The purpose of these guidelines is to provide advice and a training plan to deliver the biological sampling and tagging project modules to Pacific Islands Regional Fisheries Observers (PIRFOs) during national, regional and sub-regional training workshops. This document is your guide to:

- i. training PIRFOs in theoretical and practical techniques: (1) collecting biological samples from tuna and bycatch species, (2) reporting tag recoveries and (3) secretly deploying seeded-tags.
- ii. scheduling training according to the modules available and the projects undertaken by the Secretariat of the Pacific Community.
- iii. assessing the trainees' understanding and ability to carry out these tasks with a view to award a certificate of competency.

Training preparation

The biological sampling and tagging training is part of the overall observer course run by the lead trainer, as a team you need to discuss the schedule for delivery with him or her. You should inform the lead trainer that your schedule is based on the maximum number of fish being available. This part of the preparation needs to be done at least a few weeks in advance of the training.

The training schedule starts with the classroom sessions and then moves on to the practical work. For each activity on the schedule, refer to the relevant module in this guide for information.

You need to organise the teaching documents needed. These include the PowerPoint presentations, the printed biological sampling and tagging guides, the forms, the scenarios and the assessments. You will need to check where the most practical place to print these documents is, or who could do it for you.

The preparation for practical training, including obtaining fish heads and whole fish, securing a place to work and having the right tools, is described below. This is important and you should be prepared well in advance.

The materials needed for both theory and practical sessions are listed in the equipment check list below. It is very important that you use this check list (print it) to make sure that nothing is forgotten (particularly in terms of transporting and organising materials).

Theory teaching

Make sure that a laptop computer with MS Office is available to deliver the PowerPoint presentations. Generally, if you don't have a personal laptop, one of the other trainers might have one that you could borrow. Check in advance that the laptop will not be wanted at the same time as you want it.

Before starting a presentation:

- load the presentation onto the computer and make sure it works properly;
- make sure that the computer can be plugged into the video projector and that it works (focus and size);
- check that the videos work properly (that you have the right player) with the presentations and that slides' animations run smoothly.
- have the folder containing the videos ready so you can play the appropriate video when the slide "video" comes up. Make sure the videos illustrating the sampling techniques can be played on the computer being used.

Practical teaching

The practical teaching is dependent on the number of fish heads and whole fish which can be obtained. One of your responsibilities is to organise with the issues relevant to the practical well in advance.

- Finding a training area for the practical which:
 - is sheltered from the sun and rain;
 - has a power plug;
 - has a water supply, drains, access to a hose and can be easily cleaned;
 - is located so the work does not cause any inconvenience to others.
 - has a quiet environment to facilitate teaching guidance
- Organising the collection of enough fish heads and whole fish.
 - Make contact with fishing companies and staff of local fisheries authorities at least 1 month ahead of time.
 - In some countries it is difficult to find fish for the training; you may need to pay for the heads and/or fish. If this is the case, you should ask for receipts and file a reimbursement claim after the training.
 - Organise a freezing facility to store the fish heads and whole fish. Freezers are available with fisheries contacts at:
 - NORMA (Pohnpei); MIMRA (Majuro); Koror (Palau); Suva (Fiji) ;
 - FTC Tarawa (Kiribati) and NFC Kavieng (PNG)
 - The number of **heads** you need depends on how many trainees attend the training. **Always try to get more heads than you need to allow the trainees to get plenty of practice.**

You will need a minimum of one head per trainee for each of the three methods for otolith extraction plus one for yourself. For example: if you have 12 trainees you will need 13 small heads, 13 medium heads and 13 large heads = 39 heads. Try to get 60 !

 - The drilling method requires large tuna heads
 - The cutters method requires medium heads

- The saw method requires small heads

Table showing the head size required to train for each method. Note that the saw method can be used on any size head really, but the bigger ones should always be kept for practicing the cutters and drilling methods.

Head size for (extraction method)	Head Length		UF Length	
	<u>Min</u>	<u>Max</u>	<u>Min</u>	<u>Max</u>
Large (Drill)	30 cm	40 cm	96 cm	128 cm
Medium (Cutters)	25 cm	35 cm	80 cm	112 cm
Small (Saw)	20 cm	25 cm	64 cm	80 cm

IMPORTANT!

There are a few ways to obtain fish heads and whole fish, either from a purse seiner, a long liner or from a processing plant.

- If the head is obtained from a whole fish given by a purse seiner then time should be allocated to remove the heads and gill them so they ready to be used for practice. Trainees are not expected to do this preparatory work, especially if there is a lot of fish. If you can't to do it yourself or don't have the time, you can ask or even contract someone to do it.
- When getting your fish heads from a processing plant, ensure that they are cut correctly so that the otic capsules are not damaged. To ensure this, at least the first three vertebrae (back bones) must be included with the head. **You must make absolutely sure that this message is understood.** You could give pictures to the people who are providing the heads so that they are aware of what you need. If you are not collecting the heads yourself, make sure that you check the heads that are collected in good time to resolve any problems or, if you cannot be there yourself, get somebody who knows what is required to check.
- One **whole fish** per trainee is sufficient. When asking for whole fish, tell the provider that the fish **MUST NOT BE** gilled and gutted.
- Allow time before the practical for the heads/fish to defrost. On the day before the practical, take them out of the freezer and leave them to defrost overnight.
- Use some large rice bags to place the fish/heads in so that there are not too many flies around. Make sure the bags are placed somewhere where dogs cannot reach them.
- When in contact with local fisheries staff, never assume everything is going to be all right. Even if someone tells you: *No problems; we can do it*, keep reminding them until you can see for yourself that the heads and fish have been placed in a freezer or that there is a vessel scheduled to unload heads/fish that can be used for the training.
 - If necessary, ask your contacts to think about getting fish from the market, or you can do so when on site if no heads/fish are stored in advance.
- Organise extraction tools

- Carry your own biological sampling training kit (and keep it clean and in working order).
 - If you do not have a drill of your own, arrange for one to be lent to you in the country you will be travelling to. Organise this in advance.
 - Make an inventory of your tools after every training session to make sure they are all there and in working order.
 - When you arrive in the country, buy a can of spraying lubricant, such as WD40, to spray the tools after each practical. Note: You will not be allowed to transport these aerosol cans on the plane for security reasons.
- You also need to plan when the practical will take place. Once you know this, let the trainees know and advise them to wear old clothes that day as they will probably get dirty.

Equipment Check list

The check list allows you to make sure you have all the equipment needed for delivering the theory and practical sessions. Print a copy of this list and write OK when you have gathered the equipment or organised it. The list is based on 15 trainees attending, which is generally the maximum. If there are more than 15 trainees, increase the number of items. If there are fewer than 15 trainees, bring back any items you did not use for the next training.

<i>Items</i>	Quantities for 15 trainees	Check OK
<i>Extracting equipment</i>		
<i>Knife</i>	3	
<i>Saw</i>	3	
<i>Nail removers</i>	3	
<i>Side cutters/pliers</i>	3	
<i>Tweezers</i>	3	
<i>Power drill</i>	1	
<i>Drill tightner (if not provided with drill)</i>	1	
<i>Drill hole saw 38 mm</i>	1	
<i>Drill hole saw 44 mm</i>	1	
<i>Extension cord</i>	1	
<i>Power adaptor</i>	1	
<i>Sampling equipment</i>		
<i>Cable-tie labels (with 5 labels)</i>	50	
<i>Small bags</i>	20	
<i>Medium bags</i>	40	
<i>Large bags</i>	20	
<i>Vials</i>	16	
<i>absorbent paper</i>	1 roll	
<i>Water container</i>	1	
<i>Tagging equipment</i>		
<i>Seeding tags</i>	25	
<i>Tag seeding applicator</i>	1	
<i>Conventional tag</i>	16	
<i>Conventional tag applicator</i>	1	
<i>Teaching equipment</i>		
<i>General biological sampling presentation</i>	1	
<i>Trophic dynamics presentation</i>	1	
<i>YFT/BET reproductive biology presentation</i>	1	
<i>Tagging project presentation</i>	1	
<i>Tag seeding presentation</i>	1	
<i>Tagging project review</i>	1	
<i>Biological sampling review</i>	1	
<i>Biological sampling guide (printed)</i>	16	
<i>Biological sampling forms (printed)(scenario and practical)</i>	32	
<i>Biological sampling teaching cards (paper fish)</i>	18	
<i>Tag recovery manual (printed)</i>	16	
<i>Tag seeding guide (printed)</i>	16	
<i>Tag recovery scenarios (printed)-3 scenarios</i>	16	
<i>Tag recovery scenarios #1 correction</i>	16	

<i>Tag recovery scenarios #2 correction</i>	18	
<i>Tag recovery scenarios #3 correction</i>	18	
<i>Tag recovery forms (for scenario) (printed)</i>	50	
<i>Tag seeding scenarios (printed)-2 scenarios</i>	35	
<i>Tag seeding scenarios (printed)-corrections</i>	3	
<i>Tag seeding logsheet (printed)</i>	35	
<i>Tag recovery envelopes</i>	20	
<i>Tagging posters-English</i>	20	
Assessment equipment		
<i>Biological sampling assessment # 1 (printed)</i>	18	
<i>Biological sampling assessment # 2 (printed)</i>	10	
<i>Biological sampling assessment corrections #1 (printed)</i>	3	
<i>Biological sampling assessment corrections #2 (Printed)</i>	3	
Assessment equipment		
<i>Tagging project assessment # 1 (printed)</i>	18	
<i>Tagging project assessment # 2 (printed)</i>	10	
<i>Tagging project assessment corrections #1 (printed)</i>	3	
<i>Tagging project assessment corrections #2 (printed)</i>	3	
Hardware equipment		
<i>USB stick</i>	1	
Stationery		
<i>Red pen for correcting</i>	1	
<i>Large black permanent marker</i>	1	
<i>Highlighter pens</i>	1	
<i>Notebook for report</i>	1	
<i>plastic sleeves for attaching document on esky (if samples)</i>	2	
Transport equipment		
<i>Gel packs</i>	10	
<i>Duct tape</i>	2	
<i>Esky/cooler</i>	1	
Permits for samples collection		
<i>AQIS import permit</i>	3	
<i>NC import permit</i>	3	
<i>CSIRO letter</i>	3	
<i>SPC letter (certificate of origin)</i>	3	
<i>Keep frozen sign</i>	3	

A. Theory Component

1. PowerPoint presentations

The PowerPoint presentations are the main tools used to deliver the information about established scientific programmes. There is a general presentation, which provides knowledge of the techniques used in biological sampling. Two presentations illustrate the tagging projects. Finally, two review presentations are used as refresher before the final assessments.

Some presentations are linked to videos.

Equipment shown in the PowerPoint slides can be passed around for the trainees to look at (e.g. cable-ties, tags, forms).

To ensure you have the attention of the trainees throughout the session, give them a break at some stage. Judge their level of attention to decide when a break is appropriate.

Before the start of each presentation as well as during the presentation, encourage trainees to ask questions.

1.A General biological sampling

This presentation is an overview of biological sampling standards. It will take about one and a half hours to present.

The general biological sampling presentation shows why biological samples are needed and how they are used. Most importantly, this presentation describes how the samples are collected and how the data are recorded.

Some of the slides in this presentation are animated so that trainees are taken step by step through various protocols.

The videos linked to this presentation are:

- otolith sampling
 - saw technique
 - cutters technique
 - drilling technique
- dorsal spine sampling
- removal of the first dorsal spine.

1.B Tagging projects

Before the presentation, distribute the tag recovery forms and the tagging posters to the trainees.

This presentation shows SPC's tagging projects.

It covers the background of the tagging projects, the type of tags used and the recovery forms. Pay particular attention to the recovery form and be sure that all the data fields are explained step by step. It is recommended that you refer to *Tag recovery manual for fishing vessels /carriers* and to the page related to each subject. While the trainees listen to the presentation they can match the information with the manual.

This presentation will take about one and a half hours.

There is only one video linked to this presentation. It shows tagging action on a pole and line vessel.

1.C Tag seeding project

This presentation is about SPC's tag seeding project. It will take about one hour to present.

The presentation describes the tag seeding experience and explains its implication for the tagging project. Information is provided on the deployment of tags on board purse seiners and how to fill in the tag seeding log form.

2. Training in filling in forms

The trainees will practise filling in the various forms used to record data associated with biological sampling and tagging by means of scenarios, both in class and during practical sessions.

Tell the trainees that they should make sure to fill in the forms correctly, as you will assess them and then hand them back to the trainees with feedback on how well the forms were completed. Remind the trainees that the information needs to be written clearly.

For the tagging scenarios, if a white board is not provided for the training you can project the correct version of the forms with the video projector.

2.A Biological sampling form scenarios

This scenario is done as a group and is interactive. The objective is to familiarise trainees with filling in the biological sampling form (BS form).

This scenario is done after the general biological sampling presentation and should take about 25 minutes.

Refer to the instructions below for a detailed delivery and assessment method.



Bigeye tuna; caught at 13h03; label # B1001; length 110 cm UF; Male. Samples needed for reproductive biology studies.

Instructions:

1. Briefly mention the samples needed for the two projects: trophic dynamics (stomach, muscle and liver) and the reproductive biology studies (gonads, otoliths and spines).
2. Remind the trainees about the abbreviation for sex (Male–M, Female–F, Indeterminate–I and Unknown–U).
3. After showing the trainees how to fill in the BS form, randomly distribute the ‘paper fishes’ and one BS form to each trainee.
4. Open the animated slide where details of each fish will appear.
5. The generic information for this sampling is:
 - Observer name: The name of the trainee
 - trip #: JSM 11 00
 - Vessel name: Pacific Sunrise
 - Gear type: Longline
 - Start of trip date: 12/03/11
 - End of trip date: 25/03/11;
 - page 1 of 1.
6. In turn, ask each trainee to call out the details of each paper fish he/she has.
For example: *It is a BET caught at 01.03 pm, the label number is B1001, it measures 110 cm UF, and it is a male. Samples taken were: stomach, muscle, liver. The muscle sample was taken from the anal region.*
Note: All muscle samples should be taken from the anus, unless the fish is kept for consumption by the crew.
For the sake of this scenario, all the otoliths are sampled in port.
7. While the trainee is calling out the details of his/her fish, the other trainees fill in the information across one row.
8. Then everyone has to fill the samples that need to be collected on the BSF (muscle, liver...)
9. The trainee gives his answer.
10. Click on the slide and the details will appear.
11. Ask all the trainees to check their data and correct any mistakes.

NB: If the fish is not retained for crew consumption, then muscle should be taken from the anus region.

Emphasise that the form needs to be completed in full.

12. At the end of this training exercise, collect all the forms and assess them.
 - Check that all the information has been filled in and is correct.
 - Circle anything that is incorrect in pencil.
13. The next day or in the afternoon of the same day (depending on when the training was done) hand back the forms to the trainees and point out their mistakes.

2.B Tag recovery form scenarios

There are three tag recovery form scenarios that are done individually but corrected as a group.

The objective is to give the trainees practice in filling in a recovery form when a tag is found on board a vessel. There are three scenarios with different levels of information and different situations on board fishing vessels.

The trainees read the scenarios and look for all the details that can be used to fill in the tag recovery form. Some scenarios have more information than others. This reflects the reality in the field, where sometimes there is no information at all.

This session will take about one and a half hours. Allow 15 to 20 minutes for trainees to read and complete each scenario and ten minutes to review the answers together.

Distribute the three tag recovery scenarios (printed on one back to back page) along with three tag recovery forms which include a map (printed on three back to back pages).

Instruct the trainees to read one scenario at a time and complete one tag recovery form at a time. When they have finished the first scenario, ask them to wait for the correction before working on the next scenario. While the trainees are completing the forms, you can assist some of them individually if you think they need help.

Once they have finished entering the information for the first scenario, ask a volunteer to give his answer, write the answers on a white board, correct them if necessary and explain each answer. Do the same with the two other scenarios.

2.C Tag seeding log release form scenarios

There are two tag seeding scenarios which are done individually but corrected as a group.

The objective of this session is show the trainee how to fill a tag seeding log form when an observer is assigned to deploy tags onboard a purse seiner.

The idea is that the trainees read through the scenarios and look for all the details which can be used to fill in the tag seeding log form.

This will take about 45 minutes. Allow 15 minutes for trainees to read and complete each scenario and five to ten minutes to review the answers together.

Distribute the scenarios and the two tag seeding log forms.

Tell the trainees to read the first scenario and fill in the form. Then ask a volunteer to give his/her answers, write them on the white board, correct them if necessary and explain each answer. The trainees can then work on the second scenario and this will be reviewed in the same way.

3. Review

Two presentations are used for reviewing the theoretical component of the training.

- Biological sampling review presentation
- Tagging project review presentation

These two presentations allow the trainees to refresh their skills in order to be ready for the assessment. The review should be presented either a few hours before or the day before each assessment. Encourage the trainees to take notes where appropriate and advise them to read their guides as well.

B. Practical component

The following sections provide advice on how to organise the practical teaching. It starts with sessions on labelling, storing and packing samples. Then you will demonstrate the techniques involved in taking samples from a fish and how to do tag seeding. The trainees practise the techniques on the fish, following your step by step instructions.

Tell trainees how they will be assessed. When they finish one set of instructions, they should show you what they have done so you can assess it using the assessment checklist (see section 11. Not scored assessment) and give feedback.

The practical sessions are really useful for trainees to get a feel for biological sampling. These sessions should be interactive. You can ask questions to motivate the group and stimulate interaction.

Once you have seen that the trainees are able to deploy seeded tags, they are ready to start taking samples from a whole fish. Tell them to tag seed the fish before they start gilling and gutting it, and to let you know when they have done this before proceeding to the next step. You can thus assess each trainee on their ability to deploy a seeded tag (see assessment details).

Tell the trainees that after they have collected the samples, they are to remove the conventional and/or seeded tag from their fish. You will assess them on their ability to do this correctly.

After collecting samples from three or four fish, the trainees should place the rolled up samples in the freezer, or on a bucket of ice, or in the shade. This should be done as soon as possible so that the samples are not damaged by the heat.

The biological sampling form

Hand out one biological sampling form to each trainee. Explain that they will use the same form to write the details of each fish during each practical session. Tell them how to fill it in correctly: their name, a trip number (e.g. TUNA 11 05), the gear type from which the fish for the practical came, the start date of the practical (beginning of week) and end date of the practical (end of the week).

At the first practical session, write the date and time, and let them know the position of the training place (estimate or make one up if necessary).

Then, for each fish, they write down the label number, the species code, the length, the sex and write Y or N in the boxes for the types of samples collected.

At the start of each practical, they, write a new date and time and the new details for the different fish. Repeat for as many practicals or fish.

Ask the trainees to use the Comments column as much as possible, e.g. broken otoliths, only one gonad, broken gonads, tag number. Remind them that if they are onboard a longliner, they must note the tag number on the LL4.

Collect the biological sampling forms at the end of each practical. Check them and give them back at the start of the next practical session with your feedback.



4. Labelling and storing fish and samples

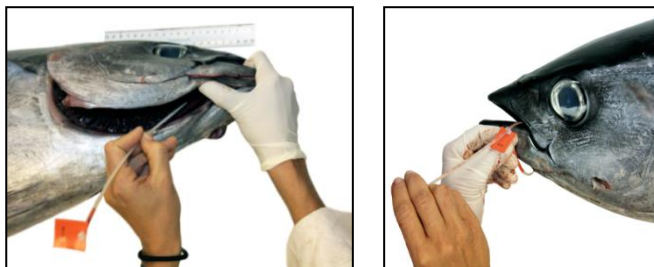
This section provides a guide to demonstrating the use of appropriate storing bags and vials for each kind of sample.

Remind the trainees that each fish is identified by a unique identification number. This means that every sample from the same fish has the same label number.

It is very important that all the samples have a label and that the labelling is done correctly to ensure there is no confusion.

4.A Cable-tie labelling

Demonstrate how to place the cable-tie around the mouth of the fish and make sure it will hold by gently pulling on it. Once the cable-tie is placed, the tear-off labels can then be used to label each sample collected. The tear-off labels are removed one at a time when a sample is placed inside a bag and needs to be labelled.



Explain why the cable-tie is placed around the mouth of the fish — when fish are landed on board and selected for sampling, placing a cable-tie around the mouth of each fish allows us to identify them. Also, otoliths cannot always be collected on board at the same time as the organs. The cable-tie will allow port samplers to identify fish that have been sampled on board from which the otoliths still need to be extracted.

The cable tie is only removed at the end, when all the internal organs have been sampled and labeled and the otoliths have been extracted and cleaned and placed inside the vial. Then the stem of the cable tie can be cut off and placed inside the vial to label the otoliths.

4.B Storing stomach, muscles, liver, gonads and dorsal spine

Demonstrate which bags are used for each kind of sample. Tell the trainees that they will need to adapt to the situation. For example, if the gonads are very big, they will need to use a large bag.

Demonstrate how to place the label inside the sampling bag at the top so that the sample does not cover the label. This allows us to read the label number when inventorying the samples later on, especially when they are frozen and bloody.

When the samples have been placed in their bags, place the largest bag on the bottom and stack the other ones on top of it. Then roll all the bags up into one package. Insist on the rule that the sample bags should not be placed inside each other.

Packaging and storing samples is an important aspect of this work,



as the samples are no use if they become damaged. Also, when samples are received for analysis, it is much easier to do an inventory if they are well packed. As with the correct way of labelling, all these steps will ensure efficient processing of the samples and, in turn, faster payment or reward.

What happens on an actual fishing trip is that, at the end of the trip, all samples are placed in a large solid plastic bag (or a rice bag) and the observer needs to inform the observer coordinator or the debriefer that there are some samples that must be stored in a freezer.

Once the observer gets back to shore, it is his/her responsibility to make sure the samples are stored in the right place and to make sure the data are attached with the samples and to make copies of or scan the data. The coordinator or the debriefer will be there to assist, but it is not their principal task.



If the observer follows all these steps, then the samples will be shipped to the place of analysis rapidly and payment will follow soon after.)

4.C Storing otoliths

Once the otoliths have been removed, clean and dry them, and place them in a vial. The vials are kept separate and do not need to be frozen. (When they go on a fishing trip, the trainees can keep the vials in a large plastic bag and keep this bag with them. At the end of the trip, the bag can be handed to the observer coordinator who will organise shipment.)



Demonstrate how to place the otoliths inside a vial and how to cut the stem of the cable-tie that is around the mouth of the fish and place it inside the vial. It has the same identification number as the other samples. Use the side cutters or a knife. Also explain how the stem can be cut to fit into a smaller vial.

Tell the trainees that this is done only after all the other internal organ samples have been collected and labelled.

5. Extraction of stomach, muscle, liver, gonads and dorsal spine for sampling

5.A Gilling and gutting techniques

First, remind the trainees what was presented during the general biological sampling PowerPoint presentation; that there are two ways of gilling and gutting tuna:

- on a purse seiner: the internal organs of the fish can be accessed by cutting the ventral surface of the fish. This must be done carefully so as not to damage the organs. Explain how to use the tip of the knife to do this. As the sampled fish will generally not be placed back inside the well, this technique can be used and performed by the observer on board.
- on a long line: the crew will generally gill and gut the fish in such a way that all internal organs are removed in one block attached to the gills. This method allows them to keep the ventral surface closed and the fish remains in good shape for marketing.

After explaining this, you have two options, depending on how many fish you have for demonstration.

If you have two fish, you can demonstrate the two techniques, one on each fish. First demonstrate the ventral surface cutting method to the trainees. This will allow them to see how the internal organs are positioned. This information is useful as they will later be examined on a picture showing the internal arrangement. Also when they sample the internal organs during the practical, the trainees will be using this method. Second, demonstrate how to gill and gut a fish as it would be done on a long liner. This will allow trainees to visualise how the internal organs are positioned using this technique

If you have only one fish, first demonstrate how to gill and gut a fish using the ventral surface cutting technique (as it would be done by an observer on board a purse seiner), then cut the gills from the mouth and remove the whole lot and place everything on the table or floor, which illustrates the long liner gill and gutting technique.

5.B Stomach sampling

Demonstrate how to identify the stomach and how to detach it from the digestive system and the oesophagus (as close as possible to the gills) using a knife. Explain that sometimes prey might fall out of the stomach and that it should be picked up and placed inside the sampling bag. In such a situation, a comment should also be provided on the form.

5.C Liver sampling

Demonstrate how to identify the liver and how to cut a sample of it (2 cm by 2 cm).



5.D Muscle sampling

On a long liner: the muscle sample can be taken around the anus area. Cut a piece of muscle next to the hole that was made during the gilling and gutting of the fish.



On a purse seiner: if the fish is going to be eaten by the crew or discarded, the observer can ask the fishing master or the captain for permission to take a sample of muscle from the back of the fish. Samples taken from this site are better as there are fewer nerves and

tendons, which allows for better analysis. The same could be done for fish with a similar fate on a long liner. However, reiterate that permission from the captain or the fishing master should always be gained before taking samples from the back.

Demonstrate how to cut a sample of muscle from the back and from the anus (2 cm by 2 cm). Demonstrate how to remove the skin from the samples.



5.E Gonads sampling

The gonads are sometimes stuck inside the body cavity near the backbone when the fish is gilled and gutted. The observer therefore needs to place his hand inside to remove them gently.

Demonstrate how to identify the gonads of a fish and how to remove them without damaging them.

5.F Dorsal spine sampling

Demonstrate how to identify the first dorsal spine of the fish and how to remove it. This technique is very easy and minimum harm is done to the fish. Once the dorsal spine has been removed, it is placed inside the bag with the gonads. Emphasise that the spine should be placed carefully so it does not pierce the bag.

6. Sex determination

Demonstrate how to visually determine the sex of a fish by looking at the gonads. Ovaries (female-F) are orange in appearance and grainy if cut. Testes (male-M) are whitish in appearance and tubular if cut.

Demonstrate how to perform the rolling test to determine the sex. Place one gonad between the index finger and thumb and try to roll it. If it rolls, it is a female. Male gonads do not roll; they may even break.



If the sex of the fish cannot be determined either by looking or

feeling, the sex is said to be indeterminate, noted with an 'I' on the data form. This can happen when the gonads are small and immature.

If the gonads are simply not seen (perhaps thrown away before you could access them) then the sex is noted as unknown, 'U'.

7. Extraction of otoliths

The extraction of otoliths is an important part of the practical teaching as the technique takes practice. There are three techniques, depending on the type vessel and the species — its size and whether it needs to be kept in good condition or not. Different sized fish are used for this training. Once the otoliths have been extracted, the trainees need to remove the membrane surrounding the otoliths, clean the otoliths using some water and dry them using a piece of cloth. The otoliths are then placed inside the vial with the stem of the cable-tie.

7.A Saw method

The saw method consist of removing the top of the head to directly access the otic capsules.

Demonstrate how to use this technique using a medium size fish first. Later on (depending on the availability of fish) small and large size fish can also be used.



This is a destructive technique and is generally used only when the fish is loined in a factory or if it is going to be eaten by the crew or discarded.

7.B Cutters method

The cutters method is a non-destructive technique and consists of accessing the otic capsule by removing bones pieces at the base of the brain, between the brain and the start of the vertebral column.

Demonstrate how to use this technique using a medium size fish. Emphasise that it is important to place the tweezers in a lateral position to reach the otoliths to avoid pushing them inside the otic capsule.

7.C Drilling method

The drilling method is also non-destructive and can be used on large fish. As this method involves the use of a power tool, explain to the trainees that they should be careful when using the drill as well as when plugging in the power cord (especially in places where there is water).



Demonstrate how to use this technique on large and medium size

fishes. Once the cores have been extracted from the fish, insist on the fact that the drill must be unplugged before removing the cores from the saw.

8. Tagging

Before the trainees' whole fish sampling practical, secretly tag some fish with conventional tags (approximately five fish out of fifteen) so that the trainees are made familiar with tags when they start the sampling.

Ask them not to remove the tag until the fish has been completely sampled.

8.A Conventional tagging

Show the trainees where a conventional tag is found on a tuna (behind the second dorsal fin on an angle).

Observers on board fishing vessels are not involved in conventional tagging, so there is no need to demonstrate how to deploy these tags. The trainees just need to know where a conventional tag can be found.

In a real case situation, if a tag is found on a fish and the fish is to be sampled, remind the trainees that they must complete the tag recovery form, and also write the tag number on the biological sampling form and on the LL-4 form if on board a longliner.

8.B Tag seeding deployment

Demonstrate to the trainees where a seeded tag is deployed on a tuna (behind the second dorsal fin on an angle). Also show them how to double tag a fish.

If you have done tag seeding previously, share your experience with the trainees. Tell them where the best place to do it is, the best time, how you reacted when a crew member saw you, and how you dealt with a seeded tag that had been found the same day or during the same trip by the crew.

Remind them about the tag seeding log form.



8.C Tag removal technique

Demonstrate how to remove a conventional tag as well as a seeded tag. Insist on the fact that the anchor must also be removed completely. Also, the fish should not be damaged during this process.

Mention that while removing the tag it may break. If this happens, it is very important is to remove the whole tag, including the anchor, and to give all the pieces to the tag recovery officer or make a note on the tag recovery form.

9. Callipers

If the use of callipers has not been demonstrated yet, demonstrate how to use them to measure a fish. If it is a tuna, UF measurement should be demonstrated. If it is a sword fish or shark, other measurements should be demonstrated (LF and TL).

Tell the trainees to round down to the nearest centimetre when recording measurements. Ask them to show you how they measure a fish and assess them on this task.

10. Tools maintenance

Tool maintenance is important. Make sure the tools are washed and dried after each use. A spraying lubricant can be used to prevent rust formation. Tools are to be used for the sampling only and should not be used for any other purpose. If an observer is asked to do some sampling, he or she will be provided with a kit which must be returned afterwards.

C. Skills assessment modules

Assessing the trainees' skills is done in two ways:

- Not scored, ongoing assessment,
During the practical, the trainees' abilities to fill in forms and to carry out various methods of sampling are assessed (not scored) while they are working, using a checklist. This allows you to give the trainees instant feedback.

The checklist should be printed according to the schedule of the biological sampling training. During each practical, use the checklist to formally assess the trainees. Each skill required during each practical is listed. During the practical the trainer should check on each trainee or group of trainees (they can work in pairs during the practical sessions) and check all the assessment criteria. Use the notation 'A' for 'able' and 'U' for 'unable'. You will see that some skills are repeated in the checklist. This allows you to check each time a practical is performed that the abilities are formally assessed. In some practicals, identical protocols need to be followed by the trainees; the check list allows you to assess the trainee's consistency in fulfilling the tasks.

- Final assessment
There are two final assessments, one for the biological sampling and one for the tagging project. These scored assessments should be planned in advance so the trainees have enough time to prepare. They are closed book assessments. For trainees who fail to reach 75% during the first assessment, a second assessment is available and can be provided.

11. Not scored assessment

Use the assessing checklist table (second tab in the BS_and_tagging_training_schedule final excel file) to assess the trainees during the scenarios and the practical sessions.

11.A Biological sampling assessment

Cable-tie label

The trainer must check that each cable-tie label placed by the trainee is not going to fall off by pulling on the cable.

This is checked before the trainee starts to take samples.

Able: the cable-tie label is placed in the appropriate area and the label is firmly attached to the fish.

Unable: the cable-tie label is inappropriately placed and/or the label is not firmly attached to the fish.

Labelling and storing and samples

Stomach, muscle, liver, gonads and dorsal spine samples

Able: Each sample from the same fish has the same identification number; the samples have been placed in the appropriate bag with the label; the labels are placed at the top of the bag and the number can be clearly read; once sampling is finished, the bags are sealed, placed on top of each other and rolled into a neat package.

Unable: Possible errors: Some samples have not been placed in the appropriate bag; some bags have no labels; the labels have different numbers; the labels are covered by the samples and the numbers cannot be read; the bags are placed inside each other; the bags are not sealed; the bags are stacked randomly in an untidy fashion.

Otoliths IMPORTANT: (*Remove the otoliths from the vial to check their quality*)

Able: the membrane is removed from the otoliths and is rinsed in water or in the mouth; no remaining blood or membrane is seen on the otoliths; the otoliths are dried before being stored in a vial; the label has been collected from the stem of the cable-tie around the mouth of the fish; if other biological samples have been collected, the label number is the same as that of the other samples.

Unable: fails to demonstrate any or all of the steps above.

Gilling and gutting techniques

Assess trainees' ability to correctly gut the fish and identify the organs without extracting the samples.

Able: fish is gutted without damaging the internal organ; organs are identified correctly

Unable: internal organs have been damaged by the knife; mistakes are made in identification

Stomach, muscle, liver, gonads and dorsal spine sampling

Assess trainees' ability to correctly sample the five organs.

Able: Trainee has correctly identified each organ; used the appropriate removal techniques; sampled in appropriate quantities:

- removing the stomach as close as possible to the gills (and picking up prey if some has fallen out – the comments section should be filled in)
- removing a small muscle sample around 2 x 2 cm (4–5cm²) and removing the skin
- removing a small piece of liver around 2 x 2 cm (4-5cm²)
- removing both gonads (if they are broken, it should be noted in the comments section)
- removing the first dorsal spine without damaging the fish muscles

Unable: Trainee fails to demonstrate any or all of the above.

Sex determination

Assess trainees' ability to correctly identify the sex of a fish.

Able: Trainee correctly identifies the sex of the fish visually or by using the Rolling test Trainee demonstrates understanding of what to write if sex cannot be determined (I).

Unable: Trainee fails to identify the sex or writes an inappropriate letter if sex cannot be determined (I).

Otoliths extraction

Assess trainees' ability to correctly extract otoliths from a fish using the following methods.

Saw method

Able: cut above the eye is straight, not too deep or too shallow; the otic capsule is exposed and the otoliths are extracted.

Unable: cut is not straight, too deep or too shallow; the otic capsule is either not exposed enough or damaged; the otoliths are not extracted.

Cutters method

Able: bone material is removed appropriately with the cutters to expose the otic capsule; tweezers are used in a parallel way; the otoliths are extracted.

Unable: the bone material is removed too fast or too deep and the otic capsule is destroyed; wrong use of cutters; wrong use of tweezers; the otoliths are not extracted.

Drilling method

Able: safety precautions are taken; angle and depth of drilling is correct; the otoliths are extracted.

Unable: safety precautions are not taken; angle and depth of drilling is inappropriate; the otoliths are not extracted.

Data recording

Each trainee is provided with a biological sampling form and is asked to write all details of the fish and samples for each practical and for each scenario during the theory teaching. At the end of the practical the trainer collects the form to assess the trainees' abilities.

Assess trainees' ability to correctly record data.

Able: all fields of the biological sampling form are filled in correctly; use of comments where appropriate; writing is readable; the label number included with the samples (including inside the vial) is the same as the one written on the BS form.

Unable: one or more fields of the biological sampling form are not filled in correctly; no use of comments where appropriate; writing is unreadable; the label number included with the samples (including inside the vial) is not the same as the one written on the BS form.

11.B Tagging project assessment

Tag implementation

The trainer must check that the anchorage of the tag is properly done by gently pulling on the tag.

Assess trainees' ability to correctly deploy seeded tags:

Able: seeded tag is inserted behind the second dorsal fin with the appropriate angle and at the right depth for anchorage.

Unable: tag is placed elsewhere than behind the second dorsal fin and the deployment angle and depth are not appropriate for anchorage.

Tag removal

Assess trainees' ability to correctly remove tags:

Able: tags are completely removed (including anchor) and the fish is not too damaged in the process.

Unable: tag is broken or the anchor remains inside flesh or the fish is damaged during removal.

Biological sampling form

This assessment is done during the practical where fish were secretly tagged and trainees are meant to notice the tags and record the tag number in the comments column of the biological sampling form.

Assess trainees' ability to record tag number:

Able: tag number is recorded in the comments column with the word 'tag' in front of it.

Unable: tag number is not recorded or the word 'tag' is not placed in front of the number.

Tag recovery form

At the end of the scenario practical the trainer collects the forms to formally assess the trainees' abilities. Formal assessment is done to check that the trainees follow the instructions and corrections given by the trainer:

Able: all fields of the form are filled in correctly: use of comments where appropriate; writing is readable.

Unable: one or more fields of the tag recovery form are not filled in correctly; no use of comments where appropriate; writing is unreadable.

Using calipers to measure a fish

Assess the trainees' ability to use the callipers to record the length of a fish.

Able: the callipers are used appropriately (for tuna, UF measurement is taken) and measurement is correct (rounded down to the nearest centimetre).

Unable: the callipers are not used correctly (e.g. the total length is measured for tuna) and/or the measurement is incorrect (e.g. rounded up and/or simply incorrect).

12. Final assessment

The final assessments are closed book assessments and you should keep an eye on the trainees. Do not staple the pages together, and ask the trainees to write their name at the top of each page. Tell them to spend more time on the questions that carry more marks. When the trainees hand in their assessments, staple the pages together and verify that the trainee's name is written on all the pages.

12. A The biological sampling assessment

BS_assessment_number1_final

Print this document double-sided and in colour. Do not staple the assessment as the trainees will need to work on the biological sampling form and the LL-4 form example.

The biological sampling assessment has three major parts.

1. Questions to evaluate the trainees' understanding of the scientific projects and the required biological samples
2. Tuna anatomy pictures to evaluate their understanding of the internal anatomy of a tuna
3. A scenario to evaluate their ability to record data efficiently.

The trainer should explain to the trainees how the assessment is set up. Rapidly go through the questions (e.g. this question relates to the type of samples needed) and explain that, for the pictures, the trainees must write the name of the organ at the base of the arrow pointing towards it. For the scenario, go through the basic idea with them. Remind them to read all the questions with care as some of the answers are provided in the questions. It should take the trainer about five minutes to explain how the assessment is set out.

The time needed to complete the assessment is around **1 hour and 15 minutes - Maximum 1 hour and 20 minutes.**

12.B Tagging project assessment

The final tagging project assessment is a closed book assessment and you should keep an eye on the trainees.

Observer_Training_Assessment#1_Tagging project

Print this document, double-sided and in black and white. Do not staple the assessment as the trainees will need to work on the tag recovery form and the tag seeding form while they read the instructions on the first page of the assessment.

The tagging project assessment biological sampling assessment has three major parts.

1. Questions to evaluate the trainees' understanding of the tagging project and the tag seeding project

2. A tag recovery scenario to evaluate their ability to record data efficiently on the tag recovery form
3. A tag seeding scenario to evaluate their ability to record data efficiently on the tag seeding log form.

Rapidly go through the questions and, for the scenario, go through the basic idea with them. Remind them to read all the questions with care as some of the answers are provided in the questions.

The time needed to complete the assessment is around **1 hour - Maximum 1 hour and 15 minutes.**

13. Reassessment

For trainees who failed to reach 75% during the first assessment, there is a second assessment which is slightly different from the first one.

13.A Biological sampling assessment

BS_assessment_number2_final

Print this document double-sided and in colour. Do not staple the assessment as the trainees will need to work on the biological sampling form and the LL-4 form example.

13.B Tagging project assessment

Observer_Training_Assessment#2_Tagging project

Print this document double-sided and in black and white.

14. Marking assessments

It takes at least eight minutes to mark one assessment, so it is up to you to figure out how much time is needed to correct all assessments. During the first tagging assessment, you can start correcting the first biological sampling assessment. The same can be done for the re-assessment.

All the correct answers have been written on separate documents for both tagging and biological sampling. Print these documents and use them as support while marking the trainees' assessments.

Use a red pen for corrections.

If the answer is correct, place a tick next to it and write the score obtained in the box next to the question.

If the answer is incorrect, place a cross next to the answer and, in a few words, provide the correct answer or circle the correct choice.

The final score is obtained by adding all the marks and converting them to a percentage. If some trainees undertake the second assessment, the final score will be the higher of the two scores obtained for each assessment.

After you have corrected both assessments, use the **BS and Tagging project Assessments scores table** Excel spreadsheet to enter the final marks the trainees have received.

The first two lines of the table have examples. Delete those examples when you have understood how to fill in the results.

Write the location of where the training took place as well as the training dates (eg. Pohnpei, April 4th to April 8th 2011).

The table is divided into several parts.

1. The names of the trainees appear on the left (first name and family name).
2. The scores obtained for the biological sampling assessment are divided as follows.
 - a. Scores obtained for question 1 to 15 (number of marks)
 - b. Scores obtained for the biological sampling scenario (number of marks)
 - c. Final score (as a percentage)

If some trainees failed to reach 75% in the first assessment, they need to take the second assessment and the scores obtained are also reported on the table.

3. The scores obtained for the tagging project assessment are divided as follows.
 - a. Scores obtained for question 1 to 4 and 6 to 8 (number of marks)
 - b. Scores obtained for the tag recovery scenario (number of marks)
 - c. Scores obtained for the tag seeding scenario (number of marks)
 - d. Final score (as a percentage)

If some trainees failed to reach 75% during the first assessment, they need to take the second assessment and the scores obtained are also reported on the table.

For each trainee and assessment, in the columns headed **Final Scores (%)**, highlight in green the scores above 75% and in red the ones below 75%.

14. A Biological sampling assessments

Questions 1 to 15 can earn the trainee up to 74 marks. The biological sampling scenario can earn him or her up to 286 marks.

Add up all the marks to get a TOTAL mark out of 357 marks.

Convert the TOTAL score to a percentage. E.g. a trainee scored 285 marks in total. Multiply $285 \times 100 = 28500$ and divide this result by 357: $28500/357 = 79.8\%$. This is the FINAL score.

The pass mark is 75%.

For correcting the biological sampling assessment, refer to the following document: **BS_Assessment_1_corrections_final**.

14.B Tagging project assessments

Questions 1 to 4 and 6 to 8 can earn the trainee up to 35 marks. The tag recovery scenario can earn him or her up to 52 marks, and the tag seeding scenario up to 26 marks. Add up all the trainee's marks to get a TOTAL mark out of 113 marks.

Convert the TOTAL score to a percentage. E.g. a trainee scored 103 marks in total. Multiply $103 \times 100 = 10300$ and divide this result by 113: $10300/113 = 91.16\%$. This is the FINAL score.

The pass mark is 75%.

For correcting the tagging project assessment, refer to the following document: **Observer_Training_Assessment#1_Tagging project correction.**

14. C Revision of assessment results

Once you have corrected all the assessments, give the trainees their test papers back and spend some time on pointing out the major mistakes made by trainees. This is a good time for them to ask questions and for you to answer them.

Present the correct answers using the video projector; each correct answer should be read and explained by the trainer.

15. Training Feedback to SPC

After training is finished, the trainers must provide detailed feedback on how the training was undertaken to Caroline Sanchez and Malo Hosken.

The aim of the feedback is:

- to keep a record of which trainees are more capable than others to perform biological sampling and tag seeding (for future reference if biological sampling and tag seeding need to be undertaken by the observers);
- to be able to improve the delivery of biological sampling and tagging modules;
- to provide you with advice and recommendations if needed.

15. A Writing a report

At the end of each day, take the time to write a 200 word narrative. This includes:

- difficulties that you may have encountered (e.g. difficulty in obtaining fish or a place to work, difficulty in demonstrating some of the techniques, difficulty in keeping to the schedule, difficulty in correcting the assessment, difficulty with some trainees.).
- Initiatives you have taken and believe have allowed to improve the delivery of the practical or the in class theory teaching.
- Relevant comments such as trainees' attendance (trainees which did not attend, etc).

15. B Training schedule

BS_and_tagging_training_schedule.xlsx

The training schedule is a list of the modules you will teach, in an appropriate order. Each module has a number that corresponds to a section in this guideline. Before the training starts, print the table and use it as a reference during the theory teaching and practical training. Before doing each activity in the schedule, refer to the module in this guide to remind yourself what needs to be done.

For each module, you need to note the date when you covered it. Fill in the **Date Done** column in the schedule.

You can enter the information into the electronic file or, if you write it on the paper version, scan all the pages and send them.

15.C Assessments

Scan and send the checklist table used for assessing the trainees' ability to perform each task.

Complete the electronic version of the score table, the one specific to the BS and tagging assessments. If no computer is available, ask someone to lend you theirs and send it by email.

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APPENDIX VI

Certificates of appreciation



Pacific
Community
Communauté
du Pacifique

Certificate of Appreciation

To *Mike Cox*

Thank you for your contribution to the Tuna Tissue Bank (TTB)

Your collaboration, participation and support allowed the collection of numerous samples necessary for scientific studies on tuna species and their ecosystem.

John Hampton ————— 12th January 2016

————— Caroline Sanchez

Chief Scientist & Deputy Director FAME (Oceanic Fisheries)

Biological sampling coordinator

