Biological Sampling manual

Guide for observers / Port samplers

Pacific Community
Communauté du Pacifique

2016

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.

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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of samples started in 2001, allowing the creation of a Pacific Marine Specimen Bank, from which samples can be withdrawn for specific scientific projects.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.

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

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 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 cm^2 – 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.

1.

2.

3.

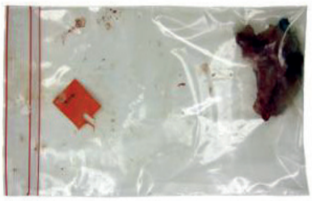

Figure 4: Sampling muscle (anal position) and placing it in a medium-sized bag.

Note:

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Liver sampling

1. Cut 4–5 cm^2 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.

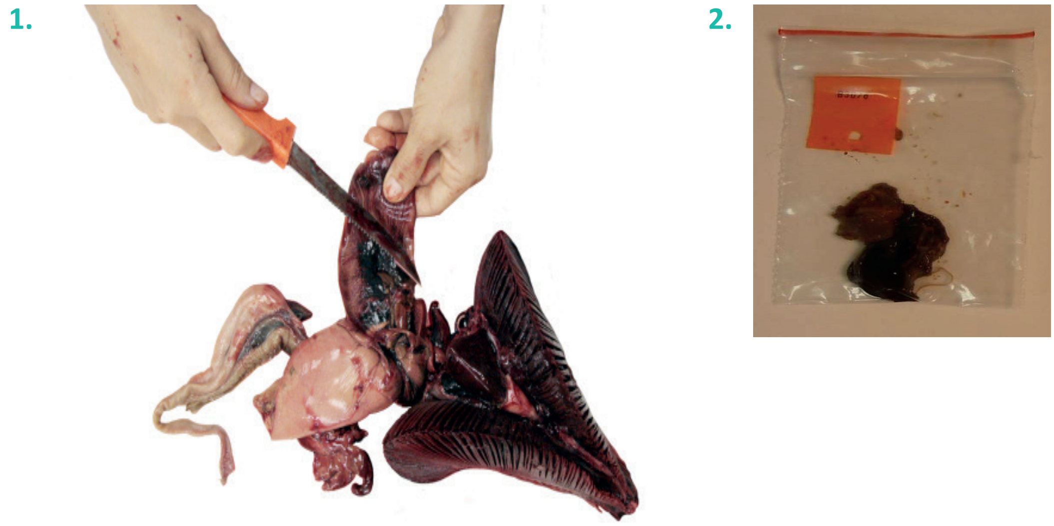


Figure 5: Sampling the liver and placing the sample in a small bag.

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.

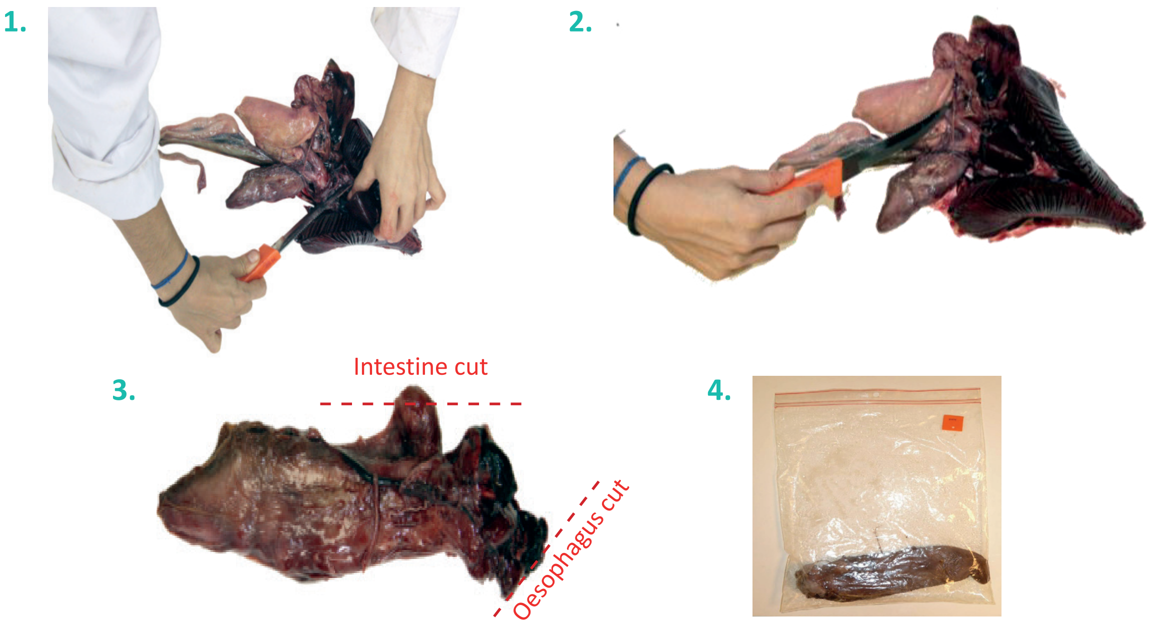


Figure 6: Stomach sampling (steps 1 to 4).

Gonad sampling and sex determination

1. Find the gonads of the fish(a): if they are not with the guts they are inside the belly of the fish(b), towards the backbone. Put your hand inside the fish to feel them. Pull out the gonads slowly; be careful not to break them. If you do break the gonads it is ok, but ensure you take all the pieces. Check inside again to ensure that you have collected all of the gonads.
2. Determine the sex. If the gonads are orange and grainy it is a female (F). If they are white and milky it is a male (M). Sometimes if the gonads are too small, you can do a test to determine the sex. Try to roll a gonad in between your index and thumb fingers. If it does roll, then it is a female. If it cannot roll then it is a male. If you cannot determine the sex of the fish by looking at it or by the rolling test, then the sex is indeterminate (I). Sometimes you may not see the gonads (e.g., they have been thrown out before you could sample them) – in this case the sex is unknown (U).
3. Place the gonads in a medium-sized bag with a label.

1a.

1b.

3.

The rolling test – this is a female gonad

2

Female gonads

Male gonads

Figure 7: Gonad sampling (steps 1 to 4) and illustrations of male and female gonads.

Note:

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.

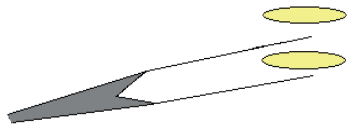
Destructive techniques:

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)



Lateral position of the tweezers

> 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.

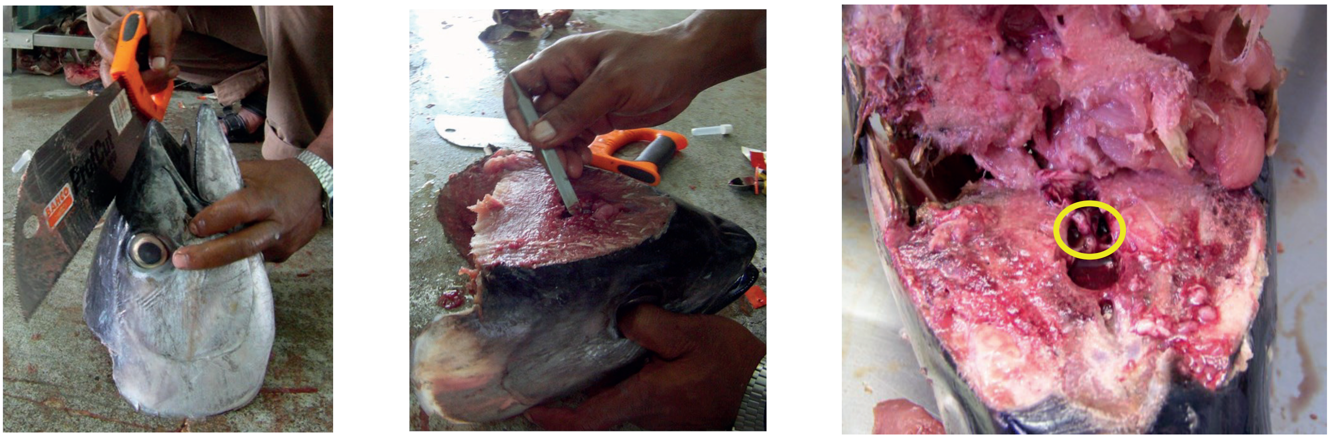
Drill cores under the gills (c)

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.

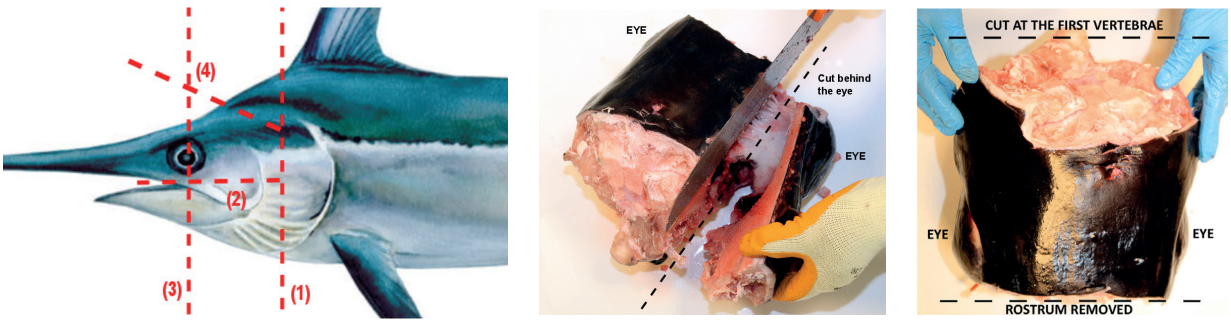
a.



b.

c.

d.



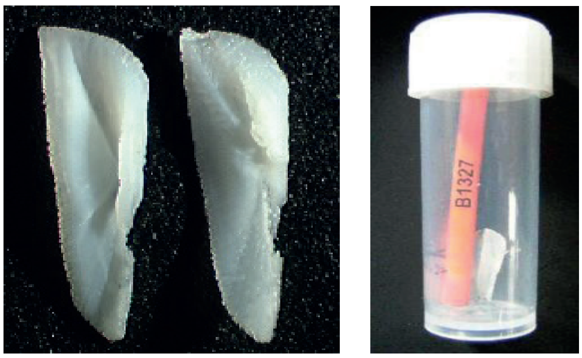


Figure 8: Extraction methods for otolith sampling using a saw, cutters and a drill. You can cut the stem of the cable tie label and place it in the jar with the otoliths.

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 time 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.

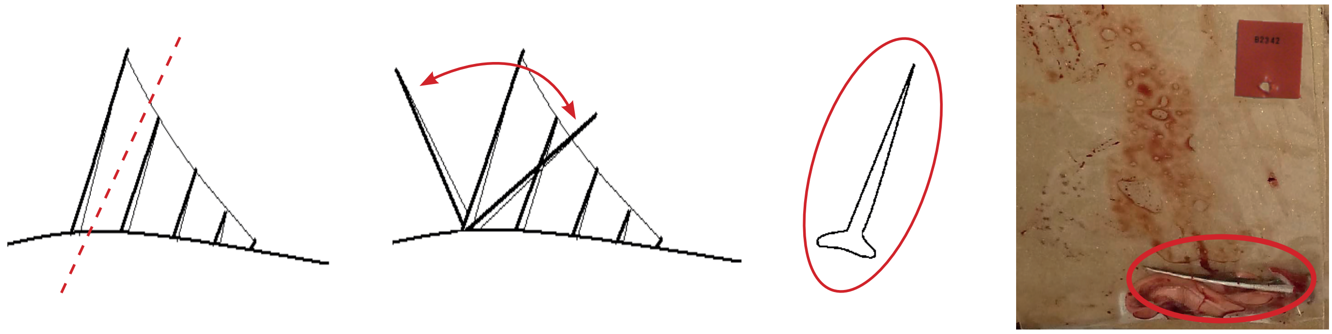
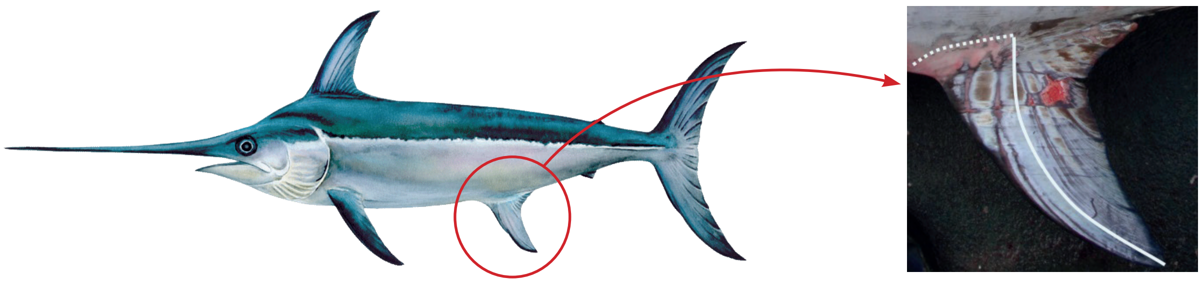


Figure 9: First dorsal spine sampling. Ensure the spine lays flat alongside the gonads.

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

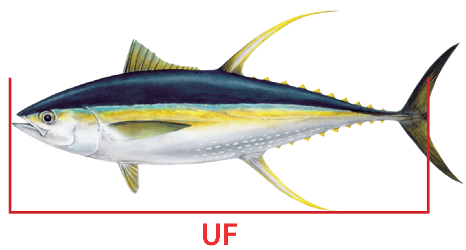
After the fish is killed, while it is still bleeding place the vial under the blood dripping from the fish. Try to fill the whole vial (a minimum of 10 ml is required). Before closing the vial place a label between the vial and the lid – while screwing the lid the label will be secured by the pressure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

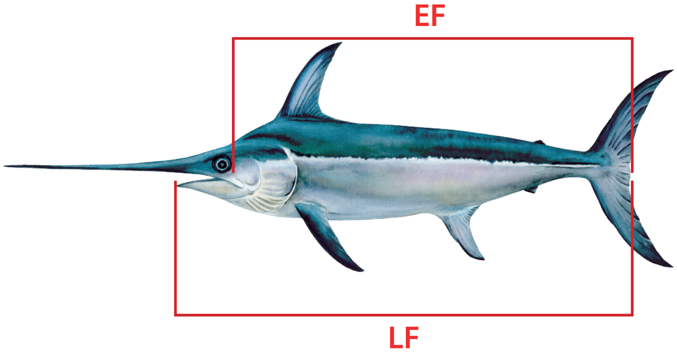
Data recording is very important. We cannot use the results from analyses of the biological samples without good data. Ensure you record the date and time, the position of the set where the fish was sampled, and its size.

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).



For swordfish, take both LF (Lower jaw to fork in tail) and EF (Posterior eye orbital to caudal fork). Note the EF in the comments section of the form. If for any reason you cannot measure the LF, 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.

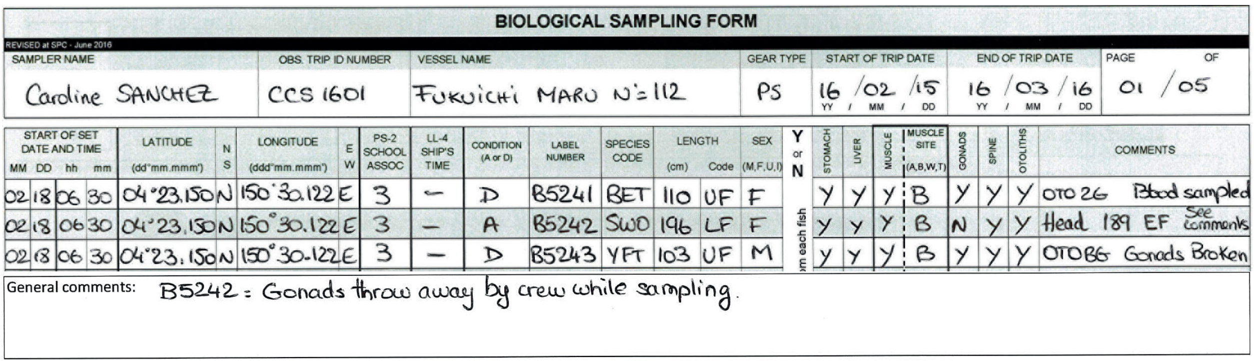
Code	Description
Oto1B	Collected only 1 otolith and the otolith IS BROKEN
Oto1G	Collected only 1 otolith and the otolith is NOT BROKEN
OtoGB	Collected 2 otoliths and 1 otolith is broken
Oto2B	Collected 2 otoliths and both otoliths are broken
Oto2G	Collected 2 otoliths and the otolith are NOT BROKEN
Head	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 (YY / MM / DD)	END OF TRIP DATE (YY / MM / DD)	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 MM	DD	hh	mm	LATITUDE (dd°mm.mmm')	N S	LONGITUDE (ddd°mm.mmm')	E W	PS-2 SCHOOL ASSOC	LL-4 SHIP'S TIME	CONDITION (A or D)	LABEL NUMBER	SPECIES CODE	LENGTH (cm)	Code	SEX (M,F,U,I)	STOMACH	LIVER	MUSCLE	MUSCLE SITE (A,B,W,T)	GONADS	SPINE	OTOLITHS	COMMENTS
02	18	06	30	04°23.150	N	150°30.122	E	3	–	D	B5241	BET	110	UF	F	Y	Y	Y	B	Y	Y	Y	OTO 2G Blood sampled
02	18	06	30	04°23.150	N	150°30.122	E	3	–	A	B5242	SWO	196	LF	F	Y	Y	Y	B	N	Y	Y	Head 189 EF See comments
02	18	06	30	04°23.150	N	150°30.122	E	3	–	D	B5243	YFT	103	UF	M	Y	Y	Y	B	Y	Y	Y	OTOBG Gonads Broken

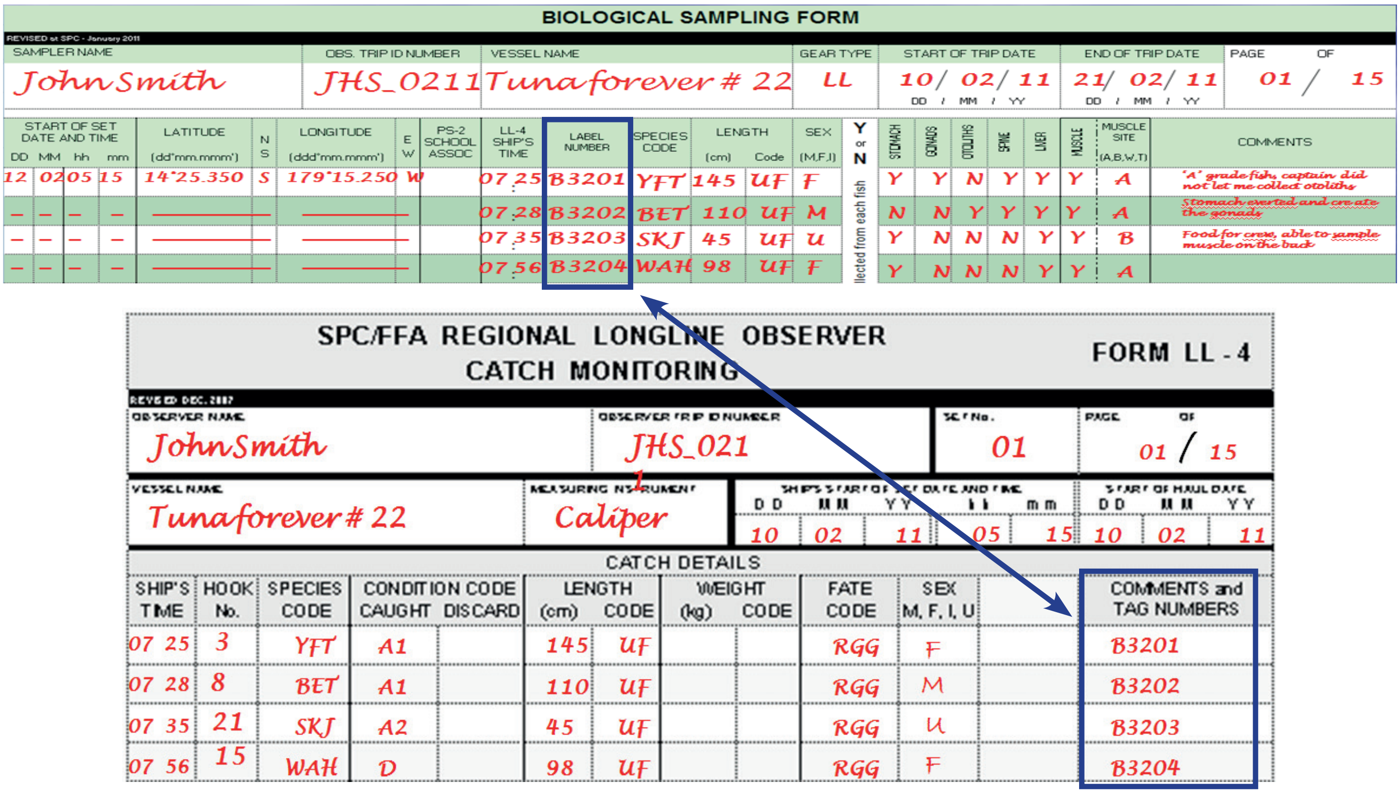
Y or N ...m each fish

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

When recording data keep in mind that all the information about the samples must be written clearly. After each sampling check your form against the samples, especially ensuring the label number used that day is identical to the samples number recorded on your form. If you find mistakes it will be easier to fix them the same day rather than at the end of the trip or during inventory in the laboratory.

Onboard a longliner: Immediately after sampling, record the label number on both forms (see example below). Then indicate what kind of samples you have collected by marking **Y**es or **N**o on the biological form. Record the location code for the muscle site. When there is time (preferably before the end of the haul) copy the LL-4 form catch details (i.e. ship's time, species code, and length and sex details) directly onto the biological form. Finally, (preferably before you start another haul) copy the LL-2/3 form set details (i.e. start of set date, time and position) directly onto the biological form.



BIOLOGICAL SAMPLING FORM

REVISED at SPC - January 2011

SAMPLER NAME	OBS. TRIP ID NUMBER	VESSEL NAME	GEAR TYPE	START OF TRIP DATE (DD / MM / YY)	END OF TRIP DATE (DD / MM / YY)	PAGE OF
John Smith	JHS_0211	Tuna forever # 22	LL	10/ 02/ 11	21/ 02/ 11	01 / 15

START OF SET DATE AND TIME DD	MM	hh	mm	LATITUDE (dd°mm.mmm')	N S	LONGITUDE (ddd°mm.mmm')	E W	PS-2 SCHOOL ASSOC	LL-4 SHIP'S TIME	LABEL NUMBER	SPECIES CODE	LENGTH (cm)	Code	SEX (M,F,I)	Y or N	STOMACH	GONADS	OTOLITHS	SPINE	LIVER	MUSCLE	MUSCLE SITE (A,B,W,T)	COMMENTS
12	02	05	15	14°25.350	S	179°15.250	W		07 25	B3201	YFT	145	UF	F		Y	Y	N	Y	Y	Y	A	'A' grade fish, captain did not let me collect otoliths
–	–	–	–	———		———			07 28	B3202	BET	110	UF	M		N	N	Y	Y	Y	Y	A	Stomach everted and ate the gonads
–	–	–	–	———		———			07 35	B3203	SKJ	45	UF	U		Y	N	N	N	Y	Y	B	Food for crew, able to sample muscle on the back
–	–	–	–	———		———			07 56	B3204	WAH	98	UF	F		Y	N	N	N	Y	Y	A	

...llected from each fish

SPC/FFA REGIONAL LONGLINE OBSERVER CATCH MONITORING — FORM LL - 4

REVISED DEC. 2007

OBSERVER NAME	OBSERVER TRIP ID NUMBER	SET No.	PAGE OF
John Smith	JHS_0211	01	01 / 15

VESSEL NAME	MEASURING INSTRUMENT	SHIP'S START OF SET DATE AND TIME DD	MM	YY	hh	mm	START OF HAUL DATE DD	MM	YY
Tuna forever # 22	Caliper	10	02	11	05	15	10	02	11

CATCH DETAILS

SHIP'S TIME	HOOK No.	SPECIES CODE	CONDITION CODE CAUGHT	DISCARD	LENGTH (cm)	CODE	WEIGHT (kg)	CODE	FATE CODE	SEX M, F, I, U		COMMENTS and TAG NUMBERS
07 25	3	YFT	A1		145	UF			RGG	F		B3201
07 28	8	BET	A1		110	UF			RGG	M		B3202
07 35	21	SKJ	A2		45	UF			RGG	U		B3203
07 56	15	WAH	D		98	UF			RGG	F		B3204

Figure 11: How to fill out the biological sampling form from the LL-4 form information

Note:

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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 IP:_________Vessel name:___________Debriefer 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 <u>BS form match the samples</u> 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 <u>individually in a plastic bag with a label</u>.
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 <u>gathered in a large plastic bag</u> 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: ____

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Pacific Community
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