Biological Sampling Manual

Guide for samplers at sea and at port

Revised by Caroline Sanchez, Giulia Anderson and Jed Macdonald

Pacific Community
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Contents

This manual is intended for scientists, observers and port samplers collecting stomach, muscle, liver, gonads, otoliths 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 and territories 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.

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 structure 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.

Studies of otolith morphology and chemistry aim to improve the understanding of population dynamics for these species by providing estimates of age, growth, population connectivity and individual's environmental and physiological histories. To achieve this, the otoliths – paired calcium carbonate "ear stones" found in the inner ear of bony fishes – are collected.

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 conducting biological sampling onboard, as well as the type of sampling to be undertaken.

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 will not be damaged.

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 (e.g. 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, therefore, are 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

Samples from each individual fish have a unique identifying number written on a label. This number is the identification (ID) number of the fish. Write down the ID number on the biological sampling form for each fish before beginning sampling.

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

Each cable tie has six tear-off labels, one of which is placed with each frozen sample. The numbered cable tie label can be used to label otoliths or dorsal spines (for marlins). The tear-off labels are mainly used for the frozen samples but, if one tear-off label is remaining on the cable after labelling them all, this remaining label can be used to label the otoliths.

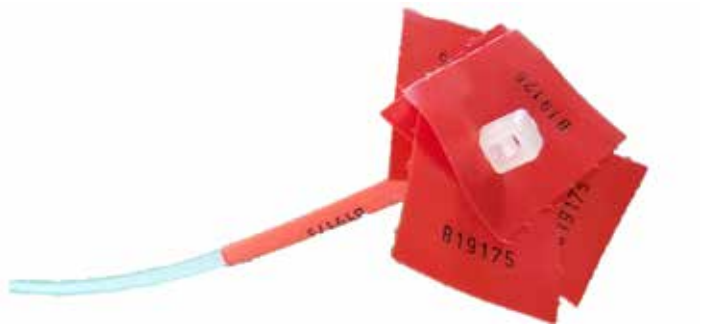


Figure 2. Cable tie ID number: All the samples from an individual fish must have the same number.

Onboard a longline vessel, the cable tie is placed through the mouth of the fish to identify the fish in port. After sampling the muscle (if not sampled at sea) and the otoliths in port, the sampler removes the cable tie with a knife, cuts the section with the label number and places it in the vial with the otoliths. The remaining tear-off label is placed with the muscle sample (if not yet sampled at sea).

At port, another type of label can be used if the sampler collects samples only at an unloading area, market or loining facility. Tear-off labels with sample types are made in strips of waterproof paper and organised in a booklet. Each strip has a unique label number starting with the letter "P".

D. Spine P-24601	Otolith P-24601	Gonads P-24601	Liver P-24601	Stomach P-24601	Muscle P-24601

Figure 3. Strip of labels for port sampling.

When you place samples in the bags, please ensure that the number on **the labels can be read.** Place the label on the 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 during debriefing or for laboratory analysis.

Prelabelled vials that correspond to cable tie numbers or port sampling labels will be provided for storing tissue samples taken with a biopsy punch tool. Additional vials with blank labels are provided in case a vial cannot be associated with the biosampling label number in use. Write the needed vial number clearly in permanent marker on the label.

Figure 4. Place the cable tie through the mouth of the fish. Ensure it will not fall off by gently pulling on it.

5

Collecting the biological samples

MUSCLE SAMPLING

> Please note: the following muscle sampling protocols are designed for the working conditions that biosamplers are most likely to experience. It is possible that samplers may deviate from these protocols in specific instances depending on storage and shipping logistics.

Depending on the species, muscle samples can be taken from around the anus, on the back of the fish, or from other parts (e.g. tail, head).

If samples are taken 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.

Basic extraction of a muscle section from the back or anus of a whole fish using a knife:

The preferred option for a muscle sample is to collect a section close to the first dorsal (4–5 cm^2, roughly equivalent to the size of an average finger). If you can collect a bigger section, it should be able to fit inside the small plastic bag.

1. Between each fish and before you sample your muscle section, clean your knife in a bucket of soapy water and wash your hands.
2. At sea, cut the muscle sample around the anus where it has already been cut for gutting the fish (4–5 cm^2, roughly equivalent to the size of an average finger). The sample section can be in a curved shape and, if it is not possible to increase the size of the cut, for example onboard a longline vessel, collect a ribbon following the cut made by the crew.

Figure 5. Sampling muscle (anal position) and placing it in a medium-sized bag.

3. In Port, if the belly of the fish is entirely removed during loining of the fish, cut the muscle around the anus area from inside the belly. Remove the skin of the peritoneal cavity first and then extract a section of muscle directly on top of the skin. The skin should stay with the rest of the belly, rendering step 3 unnecessary.
4. Always remove the skin from the muscle sample.
5. Place the muscle sample in a medium-sized bag with a label that has the number facing outwards.

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

Figure 6. The two types of muscle section (anal position) collected onboard Longline vessel.

Extraction of a muscle section from the back of a whole fish using a biopsy punch tool:

As contamination must be avoided between samples, specific equipment is provided:

- a bucket of soapy water
- nitrile gloves
- wipes, single-use
- 3-mm+ disposable biopsy punch with plunger
- 2-ml cryovials, PCR-clean with external threaded caps, filled with RNAlater and pre-labelled
- a vial box

Figure 7. The biopsy punch tool.

Specific precautions must be taken:

- Keep your hands clean and dry (no blood or fish slime) and wear the gloves. Wash your gloved hands in soapy water for 5–10 seconds before handling fish and always avoid direct contact of hands with the sampling site. Avoid touching the sampling site.
- Wash GLOVED hands in the soapy water for 5–10 seconds between handling each fish. Air dry your hands. DO NOT wipe your hands with a reusable cloth.
- Wipe the sampling location on the fish with a clean, disposable wipe. Use each wipe only once. If necessary, use multiple wipes (for fish that are bigger/slimier) until the fish skin is visibly clean and dry. Again, use the side of the wipe that your hand has NOT touched.
- Wash your gloved hand in the soapy water for 5–10 seconds before opening the sterile packaging on the biopsy punch tool.
- Do not open the biopsy punch tool or the vial until immediately before use.
- Do not touch the interior of a vial or cap with anything but the metal tip of a punch tool or the sample tissue.
- Use the vial with the same label number as other samples.
- Discard the biopsy punch tool after single use.

1. Position the biopsy punch tool at the desired sampling site (refer to the instruction provided in your sampling kit), rotate it and press down to cut through the skin of the fish. (If it is an older fish, you may need to scrape off a few scales first. You can do that with the tip of the punch tool.)
2. With the tip of the punch tool as a pivot point, swing the tool in 2–3 wide circles to sever the subcutaneous tissue.
3. "Scoop" out the tip of the punch tool. (Do not simply lift it out.)

Figure 8. Scooping the muscle sample with the biopsy punch tool.

4. Transfer the sample immediately to the cryovial filled with RNAlater. Eject the tissue sample, using the plunger function on the punch tool, screw on the vial lid, and shake the cryovial to suspend the sample in the solution.

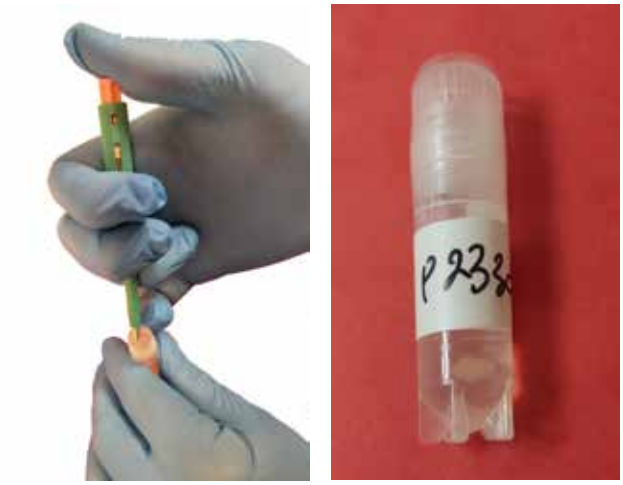


Figure 9. Ejecting the tissue sample in the cryovial using the plunger function of the biopsy punch tool.

If the chunk does not eject cleanly, you can drag the tip of the biopsy punch tool along the inside edge of the vial to coax it out. If an insufficient sample was extracted, you can use the same biopsy punch tool to extract more from the existing punch hole, as long as you are confident that nothing else has touched or compromised the tool. If you are not confident, extract additional sample using a new tool.

5. Dispose of the punch tool, immediately move the vial out of direct sunlight and/or excessive heat, ideally placing it on ice in an cooler box (esky), or in a fridge or freezer at 4°C or below.

At the end of a sampling event at sea or in port, place all vials together in the vial box and transfer to a freezer at -20°C or colder for longer-term storage.

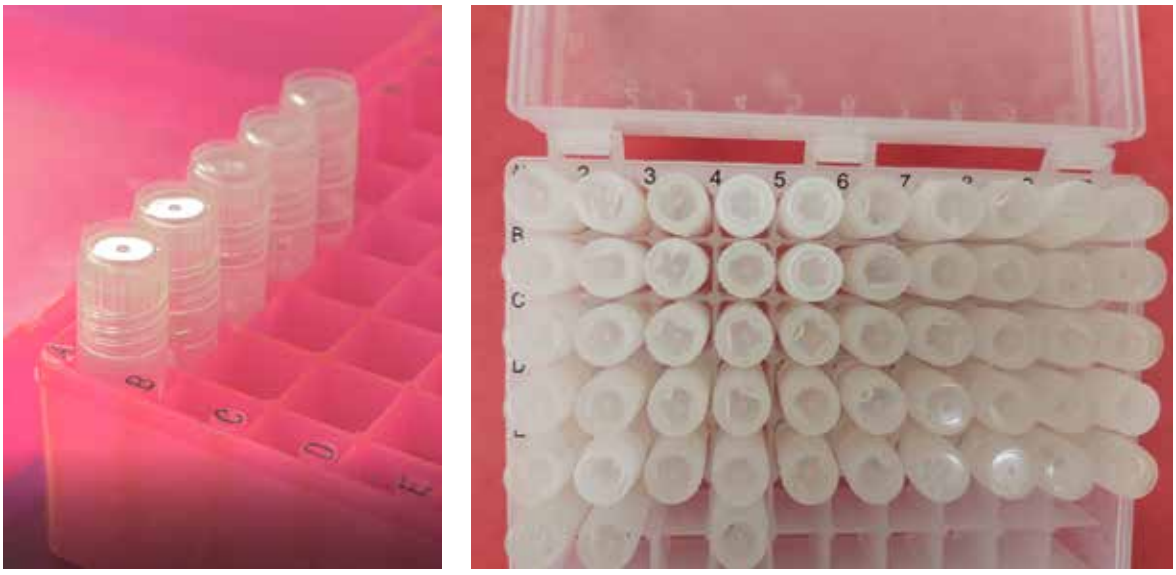


Figure 10. Vials placed in a vial box.

6. Cut a section of parafilm using scissors and seal the vial lid with parafilm. This can be done at the end of the sampling day or before disembarking.

Figure 11. Use of the parafilm to seal the vial lid.

Extraction of a muscle section from the back of a whole fish using a widget gene tagging tool:

Equipment:

- bucket of soapy water and clean wipes
- widget handles (Fig. 12)
- single-use tips loaded in a tip box (Fig. 13)
- individually labelled cryovials, PCR-clean with either snap lock or screw top lids

Specific precautions must be taken to avoid contamination of samples:

- In port, to ease the procedure, two samplers can work together. One 'fish person' to hold and manoeuvre the fish, the other widget 'sampler' who stays clean and takes the tissue sample using the widget gene tagging tool, then opens, closes and stores the cryovial containing the tissue sample.
- For widget uses, keep your hands clean and dry (no blood or fish slime). As is necessary, wipe hands dry on a clean wipe and/or wash your hands in soapy water for 5–10 seconds between handling a fish and any widget equipment, and always avoid direct contact of hands with the tip.
- If a fish is exceptionally slimy/bloody, the sampling location can also be dried with a clean wipe prior to taking the tissue sample.
- Do not open the tip box and the vial until immediately before use.
- Do not touch the interior of a vial with anything but the tip of the widget.
- Do not touch the widget tip with anything but the widget handle.
- If tips are dislodged while in the tip box (e.g., the box falls on its head while on deck), do not try to use them immediately. Use a back-up box for current sampling needs and wait until you are in a controlled environment wearing sterile, nitrile gloves to open the box and replace the tips back in their holders. If any tips fall out of the box and contact another surface, discard them.
- Be sure to use the vial with the same label number as other samples from the same fish.
- Never point a loaded widget at a person—it is spring loaded and could result in injury if discharged.

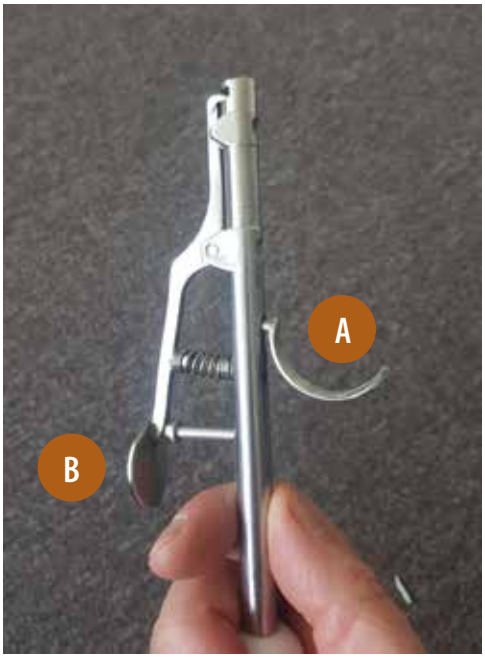


Figure 12. Widget handle components.

To load a single-use tip into the widget handle (also demonstrated in Fig. 13):

1. Pull back on the trigger with your index finger;
2. With trigger still held, push down on the thumb lever to disengage the locking mechanism;
3. Push the handle straight down onto a tip and release thumb to re-engage the locking mechanism
4. Pull the handle straight up so that the tip does not catch on its way out. Check that there is no gap between the handle and the tip and that it is fully locked into place.

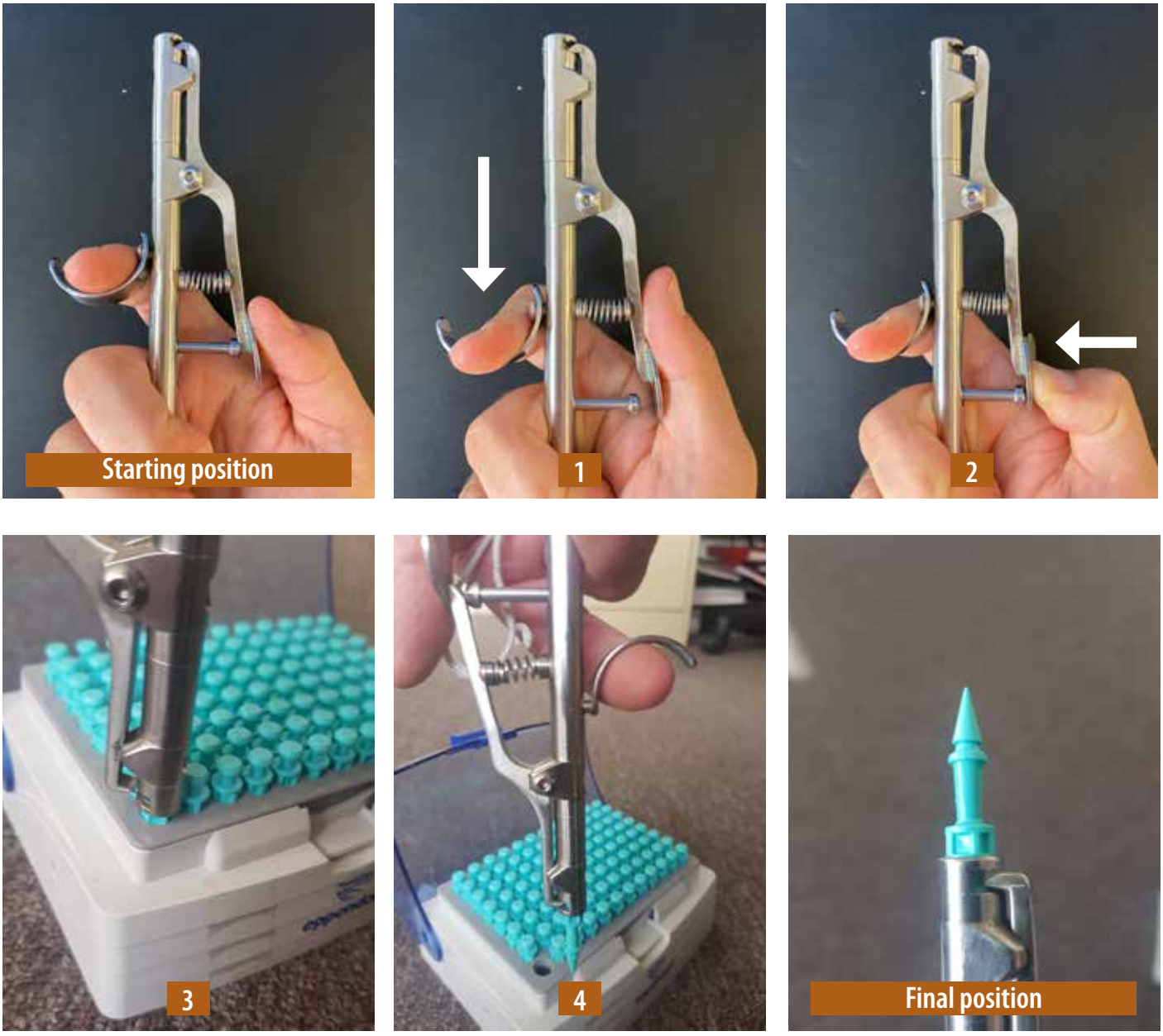


Figure 13. Push handle down on tip to be loaded. Pull straight up. Check that there is no gap between the tip and the handle.

Choosing your sampling location on a fish:

Tissue samples can be taken from a variety of positions depending on the species and the firmness of the flesh. If possible, choose a spot on the fish that has firm flesh and will not damage the fish for downstream processing. For many species, this spot is close to the dorsal fin or close to the tail. Just ensure the muscle is at least 2cm thick (e.g. not around the anus) in order to sink the widget tip deep enough to get a clean sample.

To take a tissue sample:

1. Push the tip into the flesh at a slight angle (so that it passes through several muscle bands) until it is embedded almost to the GT handle (Fig. 14).
2. Twist the handle 90° before pulling it out directly out of the fish.
3. Check the tip for flesh (Fig. 15). Ideally the hole through the tip will be filled with tissue. If there is no tissue on the tip, you can try again.

Figure 14. Taking a tissue sample using the widget gene tagging tool.

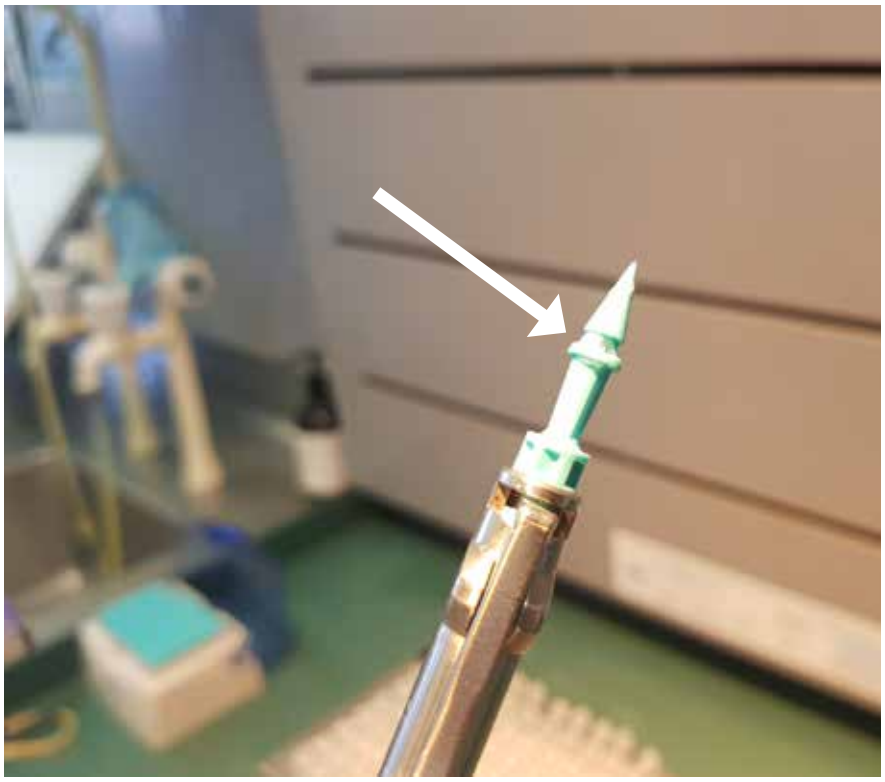


Figure 15. Checking that the tip has collected some tissue in the hole—see arrow.

Once the tissue sample has been collected:

1. Open the vial. Always use a clean hand when handling the vials and do not touch inside the vial or cap. Example of effective handling technique in figure 16.
2. Place the tip partially into the vial.
3. Pull the trigger and push down on the thumb lever to disengage the tip.

4. Immediately close the lid.
5. Place the vial in a storage box and freeze it.

The amount of tissue is small, so it is very important to store samples quickly. Depending on freezer availability and shipping options, storage instructions may vary. Most often, sample vials are filled with RNA later and are stored using the same instructions as for samples taken with a biopsy punch tool. Namely:

immediately move vials out of direct sunlight and/or excessive heat, ideally place them on ice in a cooler box (esky), or in a fridge or freezer at 4°C or below. At the end of a sampling event at sea or in port, place all vials together in the vial box and transfer to a freezer at -20°C or colder for longer-term storage.

However, alternative instructions may be provided on a case-by-case basis.

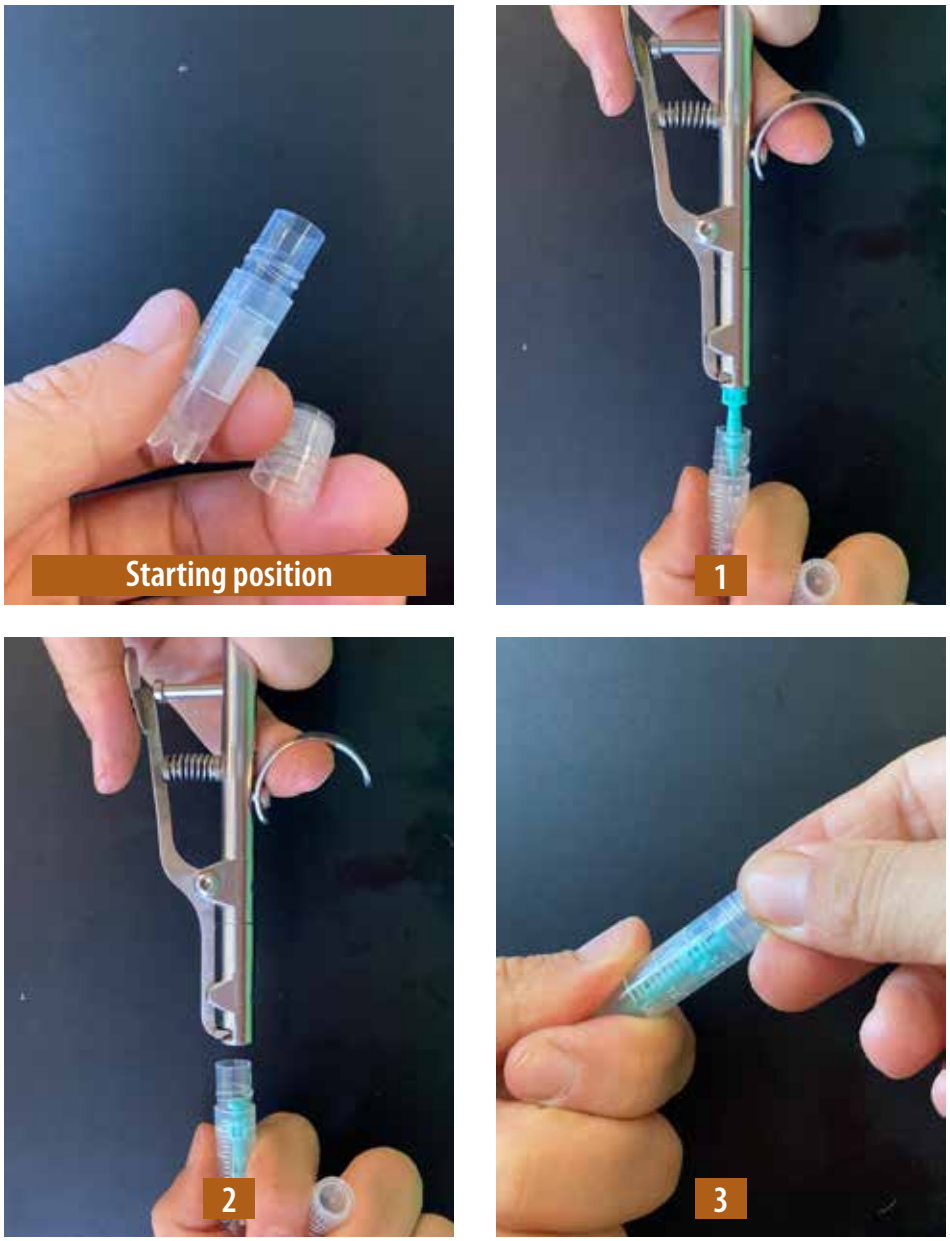


Figure 16. Loading the tissue sample into the vial.

LIVER SAMPLING

1. Cut 4–5 cm^2 (roughly equivalent to the size of an average finger) of the liver. 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.

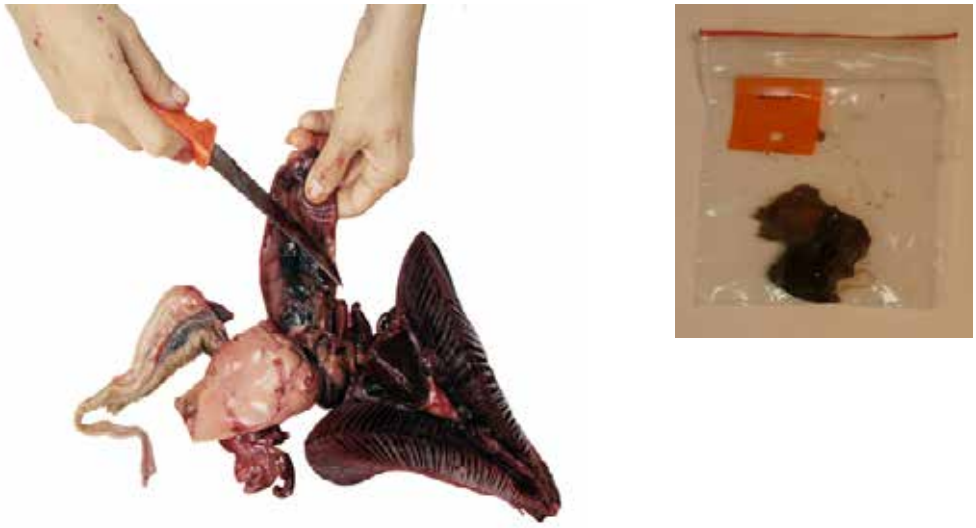


Figure 17 . Sampling the liver and placing the sample in a small bag with a label.

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 top of the sampling bag; use a drop of water to ensure the label stays in place.

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 that 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.

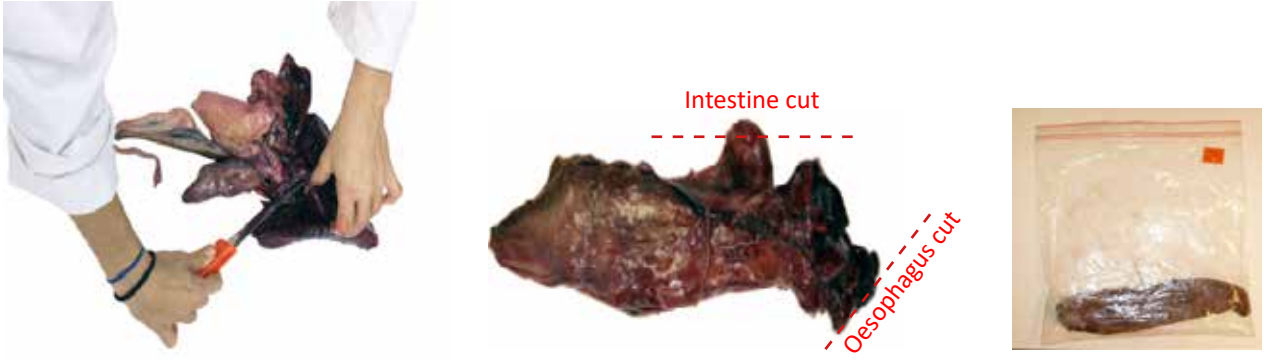


Figure 18. Stomach sampling (steps 1 to 4).

GONAD SAMPLING and SEX DETERMINATION

Gonad sampling at sea

1. Find the gonads of the fish: if they are not with the guts, they are inside the belly of the fish, towards the backbone. Put your hand inside the fish to feel them.
2. Pull out the gonads slowly; be careful not to break them. If you do break the gonads, it is okay, but ensure you remove all of the pieces. Check inside again to ensure that you have collected all of the gonads.
3. Determine the sex.
4. Place the gonads in a medium-sized bag with a label. If the gonads are too big to fit inside the plastic bag, collect only one gonad and discard the second gonad. If the selected gonad is still too big to fit inside the large plastic bag, place the gonad in the rubbish bag provided in the kit, make a knot to close the bag, and place the label just after the knot inside the remaining plastic and reseal the bag. The label is now between the two knots separated from the gonads. This will allow the debriefer or the technician at the SPC laboratory to read the label number.

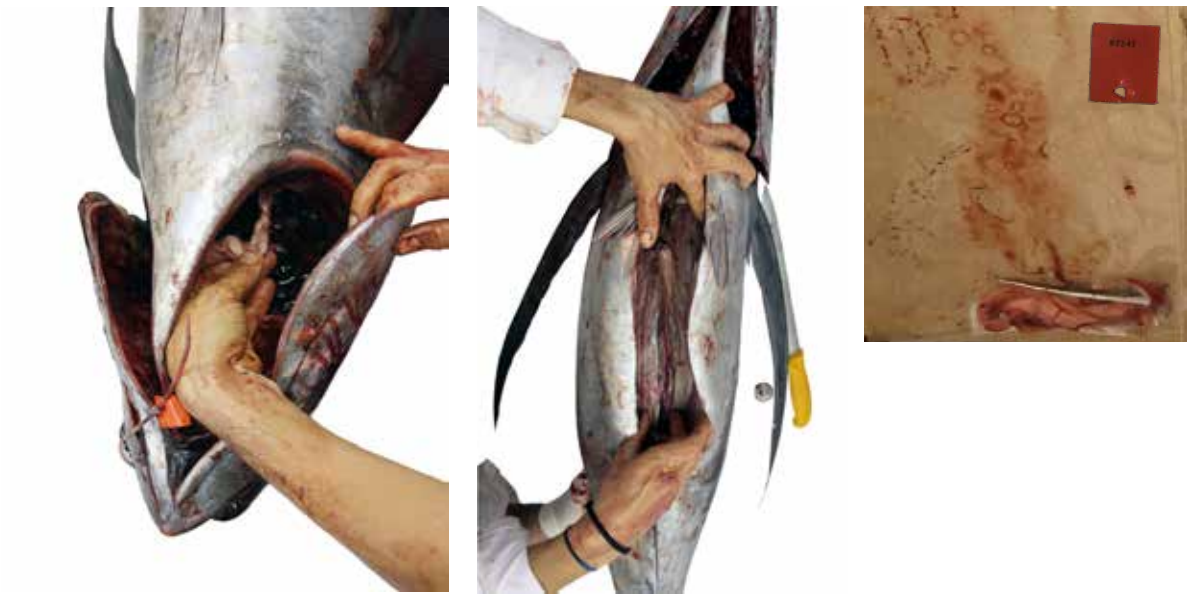


Figure 19. Gonad sampling (steps 1 to 4).

Gonad sampling at port

If you have a scale and you collect both entire gonads, **weigh both gonads.** (You can still weigh the gonads even if they are broken.) Place all of the pieces on the scale. Do not weigh the gonads if you cannot collect the entire gonad.

If you can prepare the gonad for histology analyses, follow the protocol below. If you do not have the material, freeze the gonads. If the gonads are too big, follow the same procedures as described in the instruction at sea.

For histology analyses, depending on the size of the gonads, there are two protocols:

1. If the gonads are larger than the cassette, cut a wide slice and put it in a container with 10% formalin.
2. If the gonads are small, cut a 5 mm slice (5 mm is the height of the cassette) and put it in a cassette with 10% formalin.

Always sample the biggest gonad and the largest section of the gonad. Freeze the remaining gonad section. If the gonads are particularly large, freeze the remaining gonad section that you sampled.

Formalin is dangerous: it is a combustible liquid and suspected of causing cancer. Handle it carefully and do not breathe it in.

Wear protective gloves before handling, work in a ventilated area or under a hood. Keep away from the flames and hot surfaces. Do not smoke close to the chemical. Store liquid in a store locked up and in a well-ventilated place.

Sampling big gonads

As you can see in this image, these gonads are larger than the cassette.

1. Cut a wide slice: around 5 cm wide, as the eggs need to hold together.
2. Put the slice in a 250 ml container.
3. Add 10% formalin until the formalin covers the slice (5 cm 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.)

Figure 20. Sampling big gonads.

Sampling small gonads

1. Write the sample number on the cassette with a drawing pencil. (Lightly twist the top of the cassette to remove it.)
2. Cut a 5 mm wide slice (5 mm 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 does not 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 container with the 10% formalin. (You can put several cassettes in the same container.)
5. Write the sampling date and vessel name on the container cap with a permanent marker. (Do not use a pencil.)

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

Figure 21. Sampling small gonads.

Long-term storage of the gonad samples

1. Store the container(s) with formalin in a cool place, hidden from sunlight and in a well-ventilated room.
2. After two weeks, remove the formalin from the vial and add around 70% ethanol instead.
3. On the cap of the container, remove the 10% formalin, and write 70% ethanol instead of 10% formalin.
4. Note that the formalin and ethanol are flammable liquids that must be secured properly.
5. Place the frozen gonads in a bag labelled with the country name, the sampling date and the vessel name.

Sex identification

The sex of each sampled fish must be recorded. Generally, this can be done by checking the gonads. However, the sex of sharks, marine mammals, mahi mahi and opah can be easily determined by looking at external features.

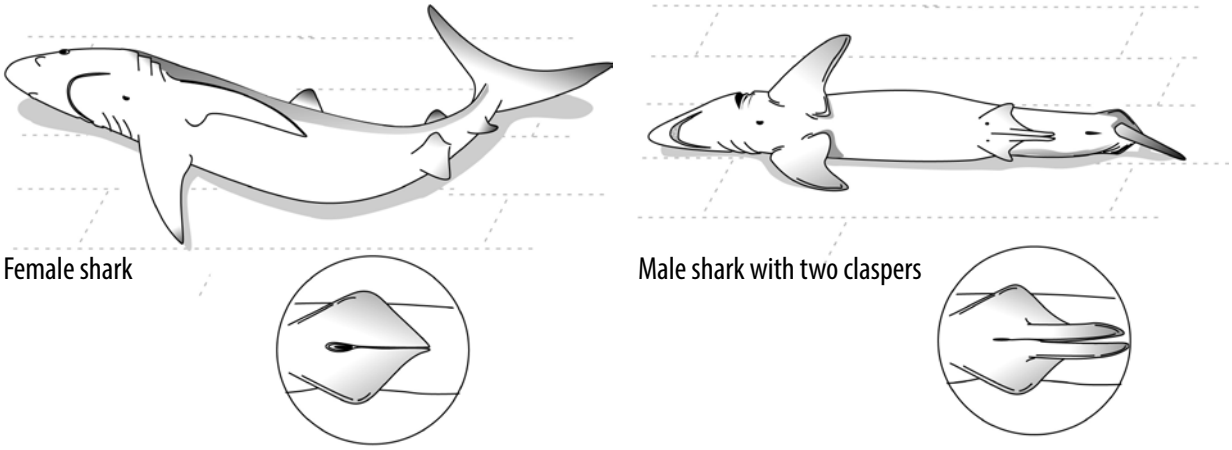


Figure 22. Sharks gonads.

For Opah, sex can be easily identified when two species are put side by side. Female "chest" area narrow with small concave outwards while male "chests" are broad and standout concave.

Figure 23. Opah (moonfish) – LAG – (*Lampris guttatus*) external feature.

Mahi mahi sex can be determined by looking at the shape of the head. The male has a straight blunt square head whereas, the female has a curved, backward sloping head.

Figure 24. Mahi mahi – DOL – (Coryphaena hippurus) external feature.

The sex of most marine species can be determined by checking the gonads, which are located inside the fish. Gonads from different species may not look the same, but they all have the same basic design. To determine the sex of the fish, locate the gonads and use the guide below.

If the gonads are orange and grainy, it is a female (F). If they are white and milky, it is a male (M). If the gonads are too small, you can conduct a test to determine the sex. Try to roll a gonad between your index and thumb fingers. If it rolls, it is a female. If it cannot roll, it is a male.

Male – "M"

A cross-section of the male gonad looks slightly triangular. It contains a lumen (small hole) that runs the full length of the gonad. The diameter of the lumen is quite small and the edges are smooth. Male gonads are likely to be white, but there may be a red tinge, depending on the maturity of the gonad. If the gonad is lightly squeezed, a white liquid (semen) may emerge. No granules can be seen when looking closely at the tissue of male gonads.

Figure 25. Lateral view and cross section of a male gonad.

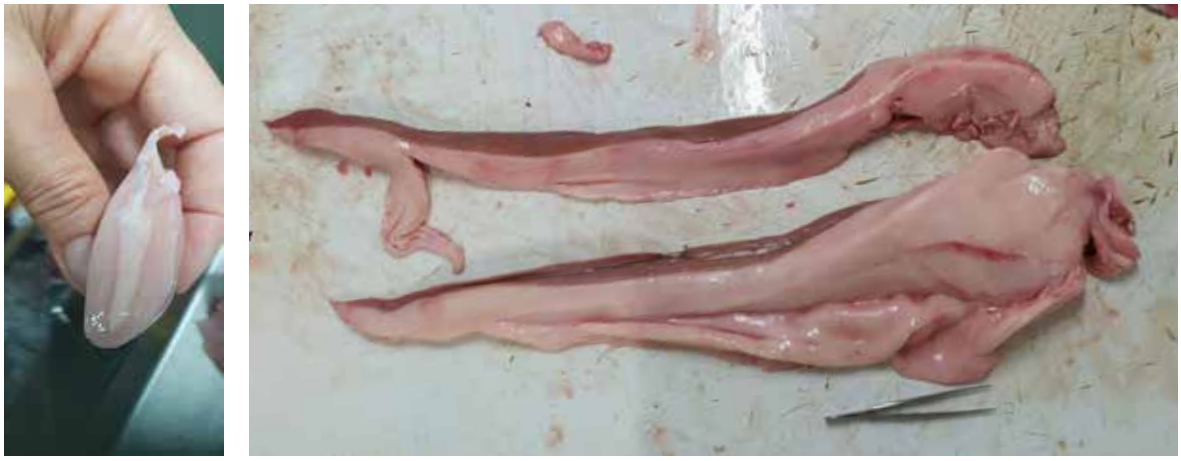


Figure 26. Male gonads.

Female – "F"

A cross-section of the female gonad looks mostly circular. It also contains a small lumen (hole) that is somewhat rough at the edges and runs the full length of the gonad. Female gonads usually, but not always, have a yellow-orange tinge. The colour may be deeper, depending on the maturity of the gonad. When looking closely at female gonad tissue, small granules (eggs) can be seen. These are more obvious in more mature gonads.

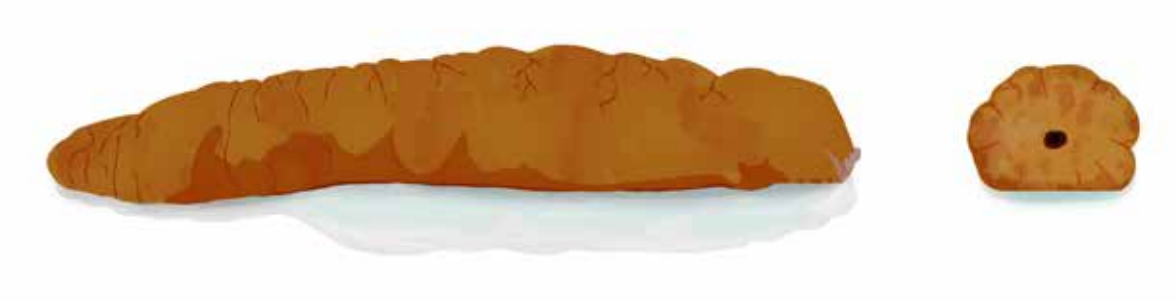


Figure 27. Lateral view and cross section of a female gonad.

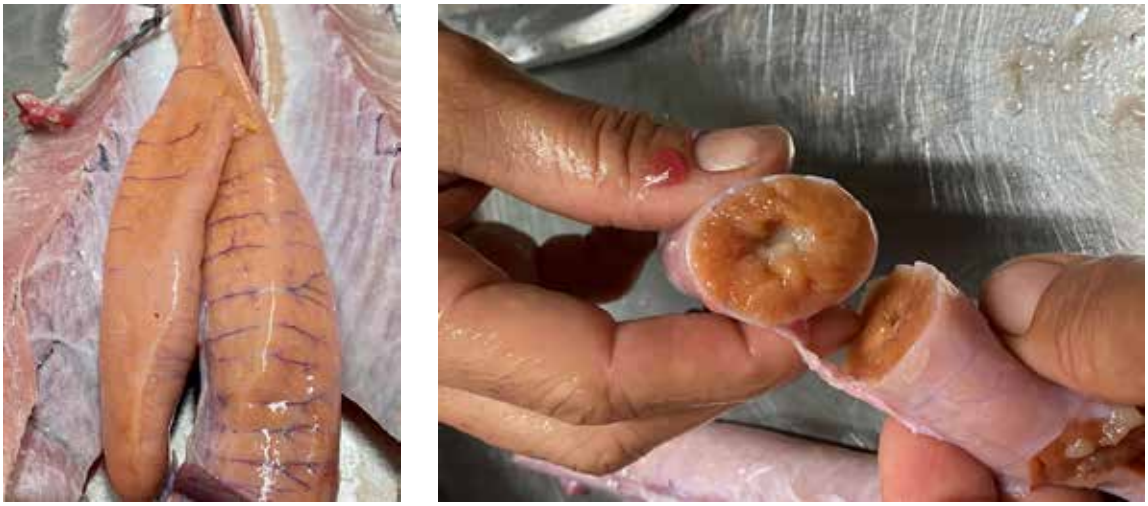


Figure 28. Female gonads.

Indeterminate – "I" (indeterminate)

If the gonad is checked but is too immature to determine the sex, the observer can record "I" – (indeterminate). Both immature male and female gonads are likely to be string-like and thin and some of the features outlined above may not be obvious when the gonad is examined.

Unknown – "U"

Use the sex code "U" – (unknown) when unable to check the sex of the marine species.

Caution: Note the difference between the sex codes "I" and "U".

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 the entire head (for very small tuna, small dolphinfish, swordfish and marlins) and leave it 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 from that of bone. If you break the otoliths, keep all of the pieces and place them together in the vial. (Refer to chapter 6 on 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.

For tuna and wahoo, 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 there is no trace of blood before placing the otoliths inside the vial. Do NOT freeze them.

For dolphinfish, do not remove the otoliths from the membrane. The otoliths are too fragile and will break. Place the otoliths with the membrane in the vial with the cable tie label. Do NOT freeze them.

Removing the top of the head technique

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, face the head towards you. Remove the brain with the back end of the tweezers.

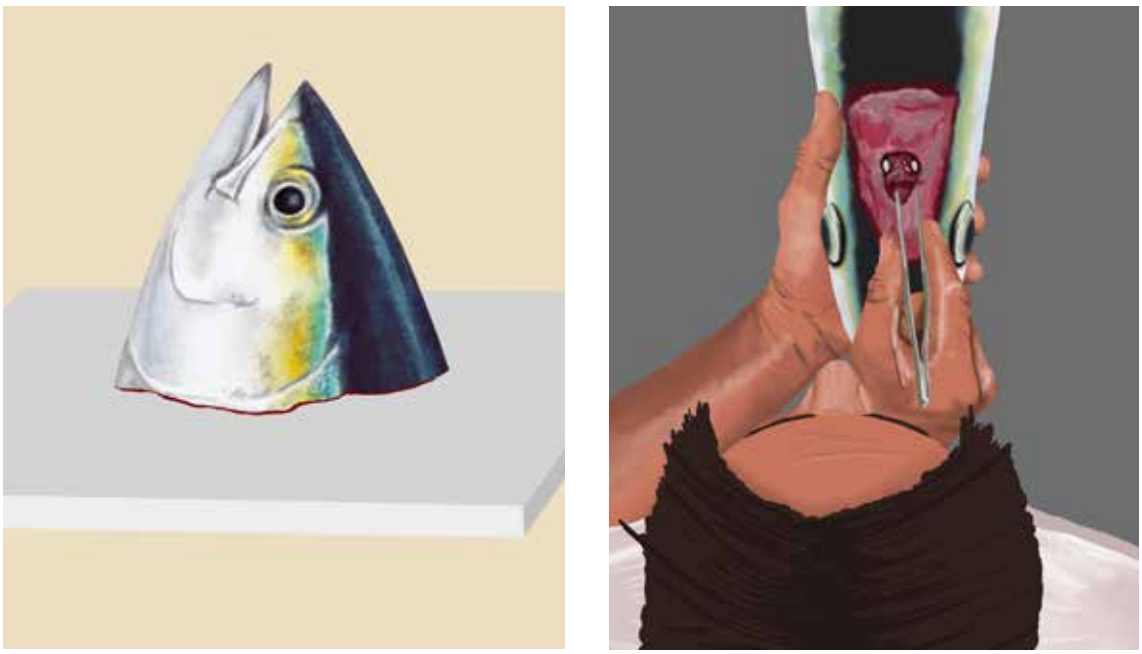


Figure 29. Removing the top of the head of a tuna to extract the otoliths.

1. Cut the otic capsule using cutters

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.

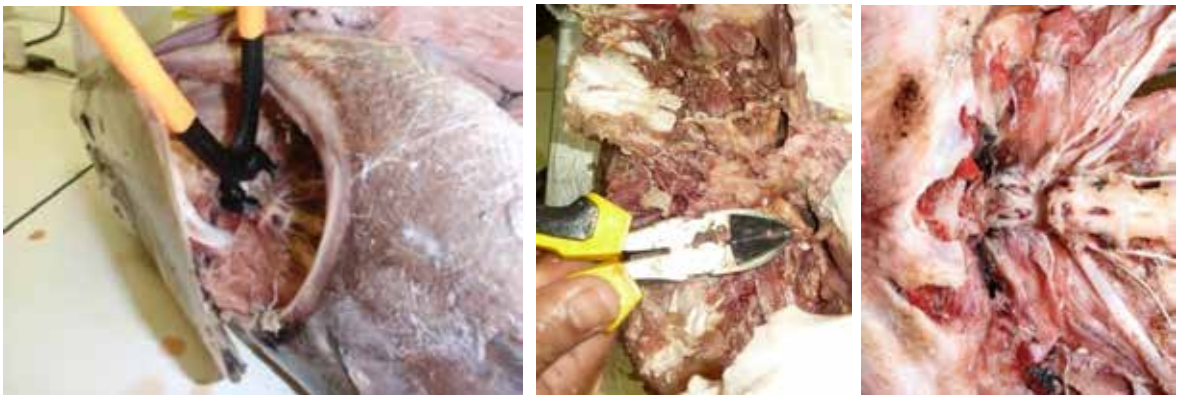


Figure 30. Removing the otoliths using cutters.

2. Drill cores under the gills

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 (ensuring that the safety lock of the drill is on). Locate the otoliths inside the bone.

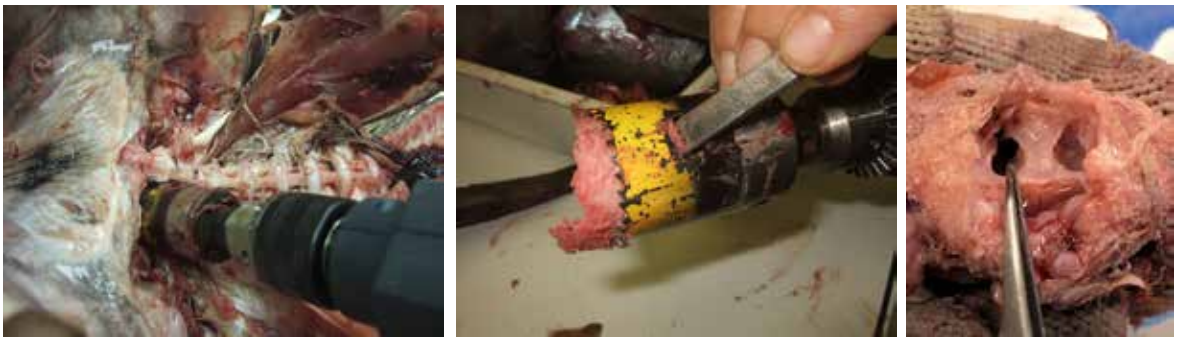


Figure 31. Removing the otoliths using a drill.

Figure 32. Clean otoliths with the cable tie label placed in the jar.

3. Remove the entire head (from small tuna, small dolphinfish, swordfish and marlins)

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. Place the cable tie label through the mouth or place the head inside a large plastic bag or a rubbish bag. Place the label in the plastic bag or if you use a rubbish bag, use the same technique as described for the large gonads (See Section 4). Remove as much air as possible from the plastic bag.

For swordfish and marlins, after you remove the head from 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.
5. Repeat for the other side. These cuts aim to reduce the sample size before the packaging.
6. Place the label from the cable tie with the head, as you will use the cable tie to close the bag with the dorsal spines or the anal rays.

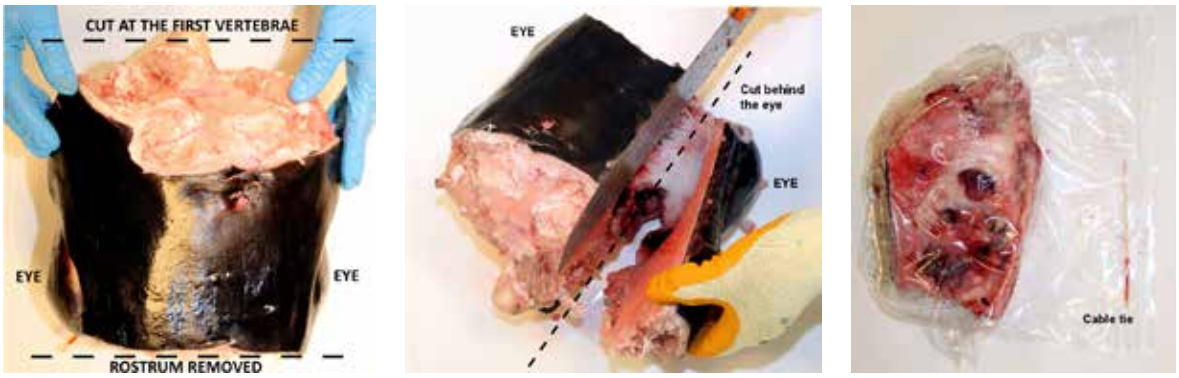


Figure 33. Removing the entire head of a swordfish.

DORSAL SPINE SAMPLING

For tuna:

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, ensuring it lays flat to prevent it from piercing the bag.

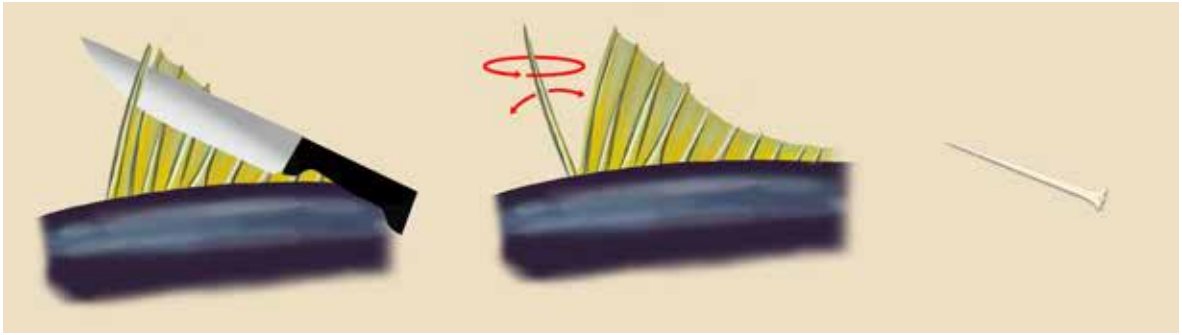


Figure 34. First dorsal spine sampling.

For marlins:

1. With your knife, remove the skin to see the pterigiophores, the spine structure that supports the dorsal fin. That will help you to see the base of the spines, the condyle.
2. Move the spines to easily see the base of the spine.
3. With your knife and your fingers, try to find the membrane separating each spine. When you find the first membrane, cut through it upwards with your knife and try to find the next spine.
4. Once you have separated them with the knife, further separate them by moving them with forward and backward movements.
5. Then with the knife, dissociate the wide base of the spine from the rest of the body. Do not cut through or damage the base.
6. With the knife, cut the tendons that retain the base.
7. Remove each spine one after the other, moving towards the front of the head and sideways to loosen the base of the spine.
8. Place the spines in a large plastic bag, with the base of the spine at the bottom of the plastic bag. It is okay if the spine sticks out of the plastic bag. Roll the plastic bag around the spine.
9. As you have already collected the head and used a small label to number the head, use the cable tie label to close the bag.

Figure 35. Extraction of the dorsal spines of a marlin.

SECOND ANAL FIN RAY SAMPLING – For Swordfish only

1. Touch the fin with your finger to identify the second ray.
2. To separate the second fin ray from the third ray, after the second ray, slide your knife upward toward the body of the fish
3. Remove the base of the fin ray embedded in the flesh by cutting under the base of the ray towards the front.(See the dashed line on the photo of the cutting guide.)
4. Place your rays in a large plastic bag. It is okay if the rays stick out of the plastic bag. If it is the case, roll the plastic bag around the rays and, as you have already collected the head and used a small label to number the head, use the cable tie label to close the bag.

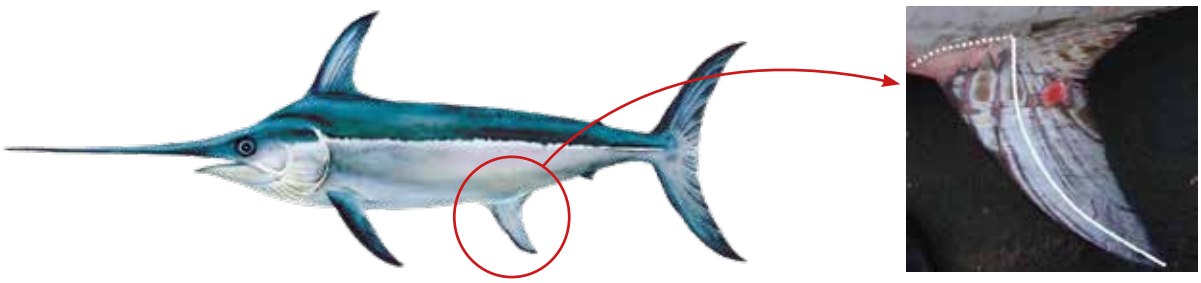


Figure 36. Extraction of the anal fin rays of a swordfish.

BLOOD SAMPLING

1. 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).
2. 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.
3. Gently pull on the label to ensure it will hold.
4. Store the vial in a freezer.

Figure 37. Blood vial with a label between the vial and the lid.

6 Recording data on the biological sampling form at sea and in port

Data recording is very important. We cannot use the results from analyses of the biological samples without reliable data. The information on the biological sampling form must be linked to the numbered labels, which will be placed in the sampling bags or the vials containing the samples. Therefore, do not forget to write the label number on the form. All information about biological samples is recorded on the **biological sampling forms**.

For purse seiners, use this form only. For longliners, match the information on this form with the information on the LL-4 form. For sampling at port or in a cannery, a port sampling form is also available.

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 purse seiners only); ship's time (for longliners 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.

When recording data, keep in mind that all of 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.

SPC BIOLOGICAL SAMPLING FORM – Onboard fishing vessel

Sampler name

The sampler must write her/his full name, as it appears in her/his passport, on every single form. Put the first name first and the last name last. Do not abbreviate the name on any of the forms.

Observer trip ID number

Fill in the complete *observer trip ID number*, as issued by the observer programme and/or the providers who have authorised the placement, or as determined by the number of trips completed by the sampler during the year. Observer trip ID numbers are individual trip codes – a three-letter staff ID code. It is assigned to all observers or samplers followed by a space, two digits indicating the year of the trip, dash and trip number (the current trip number based on the numbers of trips made by the sampler during the calendar year).

Vessel name

The name of the vessel is the name written on the fishing licence or permit, which is issued by the country to the licensed vessel. Do not abbreviate the name. Include all numbers associated with the name.

Gear type

For the type of gear used by the vessel, write "LL" for longline, "PS" for purse seiner, "PL" for pole-and-line, "T" for trolling, "VL" for vertical longline and "HL" for handline.

Trip Start (ship's date)

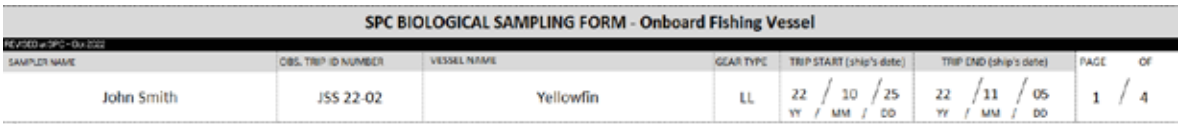
Use the ship's time to record the date that the trip begins. Record the date the vessel first casted off (threw its ropes) or started its engines to return to the fishing ground.

Trip End (ship's date)

Use the ship's time to record the date that the trip begins. Record the return date as the date the catcher vessel comes alongside the wharf or drops its anchor.

Page of

The total number of pages of biological sampling forms used during the trip. If there are five pages in total, each page should be recorded as page 1 of 5, page 2 of 5, page 3 of 5, etc.



SPC BIOLOGICAL SAMPLING FORM - Onboard Fishing Vessel

SAMPLER NAME	OBS. TRIP ID NUMBER	VESSEL NAME	GEAR TYPE	TRIP START (ship's date)	TRIP END (ship's date)	PAGE OF
John Smith	JSS 22-02	Yellowfin	LL	22 / 10 / 25 YY / MM / DD	22 / 11 / 05 YY / MM / DD	1 / 4

Fish Nb.

The number of the fish sampled for which data is recorded on the biological sampling form.

Start of set date and time

At the start of every set, fill in the "ship's date and time". Onboard longline vessel: this is the date and time that the first radio buoy/float is thrown into the water. Onboard purse seine vessel: this is the date and time the set is deployed, indicated by the captain shouting "let go", the pelican hook is then released and the skiff slides into the water. This should be the exact date and time that you have recorded for this set on your LL-2/3, or PS-2. Onboard artisanal fishing vessel: this is the date and time that the fishers deploy their gear in the water.

Latitude and longitude

Latitude and longitude positions can be recorded from the GPS of the vessel. Record the latitude (dd°mm.mmm) and the longitude (ddd°mm.mmm) to three decimal minutes. Remember to always record the direction: N (North) or S (South) for latitude and E (East) or W (West) for longitude. Onboard artisanal fishing vessel: if a GPS is not used during the fishing activity, provide the DCP's position or the position of the centre of the fishing area.

PS2-School assoc.

Record the school association code only onboard a purse seine vessel; otherwise, place a dash in this field. The presence or absence of a particular type of floating object with a tuna school is known as its "school association". See below the school association code; further description refers to the Purse-seine Observer Guide (2021).

School association code	Description
1	Unassociated (free school)
2	Unassociated (free school) with baitfish
3	Drifting log, debris or dead animal
4	Drifting raft, FAD, payao
5	Anchored raft, FAD or payao
6	Live whale
7	Live whale shark

LL-4 Ship's time

Record this information only when you are onboard a longline vessel or an artisanal fishing vessel; otherwise, place a dash in this field. This time corresponds to when the fish is brought onboard the fishing vessel. Onboard a longline vessel: this should be identical to the ship's time you wrote on the LL-4.

Condition

Record the condition of the fish when brought onboard the fishing vessel: A for Alive or D for Dead. No need to record the intermediate code (e.g. A5 or D3).

Label number

Record the number present on the label that will be placed with the sample. There is no need to record the dash between the letters and number. For example, if the label is P-12345, record P12345. All of the samples from the same fish share the same label number.

Species code

Record every species with the three-letter FAO species code. These codes are marked in the "Marine species identification manual for horizontal longline fishermen" or the "Fish species identification manual for deep-bottom snapper fishermen".

FISH Nb.	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
1	10	26	06	15	10°20.020	S	175°225.125	E		11:20	A	B12346	YFT
2	10	26	06	15	10°20.020	S	175°225.125	E		12:20	D	B12346	WAH

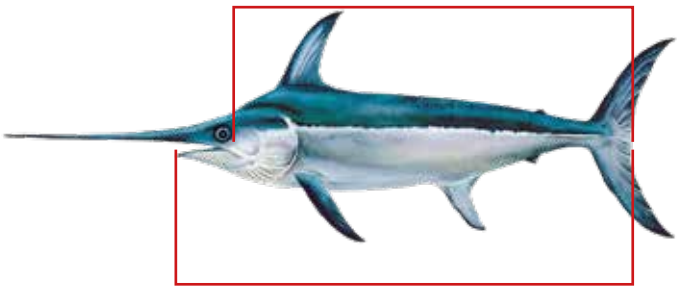
Length

Length measurements are always **rounded down** to the nearest whole centimetre. For example, if the length of the fish is 71.8 cm, record "71 cm" on the data sheet.

For whole fish, take a UF code measurement (upper jaw to the fork in the tail).

UF

EF



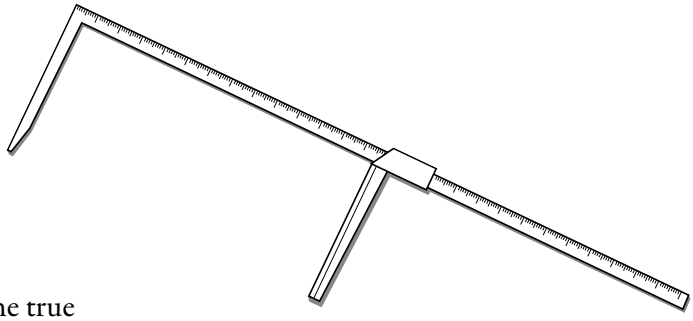
LF

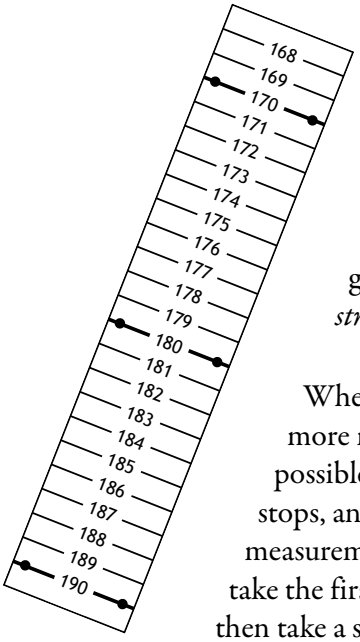
For swordfish and marlins, note both the LJFL (Lower Jaw to Fork Length) and OFL (Eye Fork Length). Note the OFL in the comments section of the form. If, for any reason, you cannot measure the LJFL, explain this in the comments section. (See the example below.)

Do not sample a damaged fish as the measurement will be approximate.

Always aim to measure fish using calipers. Calipers give the most accurate and reliable results. Understand how to use calipers correctly. Calipers are designed so that the groove on the fixed leg of the caliper is placed on the snout/upper jaw of the fish and not on the fork of the tail. Calipers must be calibrated regularly to ensure they measure accurately. To calibrate calipers, measure a section of a deck tape or ruler with the calipers. Measure 25 cm on the deck tape, then check that the calipers read 25 cm exactly. If a deck tape or a ruler is not available, use the ruler on the caliper to mark out a set length (e.g. 80 cm) on the deck. Then calibrate the calipers against the set length.

If a deck tape is used, make sure it is placed under the fish and that the start of the deck tape is placed up against a raised hard object. This will ensure that the nose of the fish is aligned with the zero mark on the deck tape. If this is not done, the fish can easily slip down the deck tape when it is being measured, giving an incorrect measurement.





Pay attention to collecting the true measurement when using deck tape. The observer's eye must be directly above the tail of the fish to ensure the correct measurement is recorded. If the measurement is taken when the observer's eye is not directly above the tail of the fish, the measurement will be read at an angle, possibly giving an incorrect result. *Always place the deck tape up against a straight (90 degrees) vertical object.*

When a fish is longer than the 1.5 m calipers, measure it by taking two or more measurements. One method is to first measure as much of the fish as possible, make a light mark on the fish at the point where the measurement stops, and then take a second measurement from that point. Adding the two measurements together gives the length of the fish. Another method is to take the first measurement at 100 cm, lightly mark the fish at this point, and then take a second measurement from the point. It is then easy to add the two measurements together to get the total length.

Sex

The sex of each sampled fish must be recorded. Generally, this can be done by checking the gonads. See section X on gonads extraction and sex identification.

Sex code	Description
M	Male
F	Female
I	Indeterminate
U	Unknown

Otoliths

Record "Y" for yes, if you collected both otoliths or only one otolith or if you removed the head of the fish for collection of the otoliths in a laboratory or at a port. Record "N" for No, if you did not collect the otoliths or if you placed a cable around the mouth of the fish for collection of otoliths in a laboratory or at a port.

Otoliths code

Record the otoliths code as described below:

Code	Description
1B	Collected only 1 otolith and the otolith IS BROKEN
1G	Collected only 1 otolith and the otolith is NOT BROKEN
GB	Collected 2 otoliths and 1 otolith IS BROKEN
2B	Collected 2 otoliths and both otoliths ARE BROKEN
2G	Collected 2 otoliths and the otolith are NOT BROKEN
H	Collected the head instead of the otoliths

A broken otolith is an otolith with the tip of the otolith broken or an otolith broken in several pieces.

Muscle

Record the technique used to collect the muscle. If you use a knife, note "K". If you use a widget, note "W". If you use a Biopsy Punch, note "P". If you use both techniques, use two lines in the biological sampling form. If you collect only one muscle sample, place a dash in the second set of fields. See the examples below.

Muscle site

Record where the muscle sample was collected from the fish. If you collect the muscle from the anal area, write "A". If it is collected from the back near the first dorsal fin, write "B". If it is collected from the head, write "H". If it is collected from the tail of the fish, write "T" and, if it is collected from any other part of the fish, specify the code on your form.

LABEL NUMBER	SPECIES CODE	LENGTH (cm)	LENGTH Code	SEX (M,F,U,I)	OTOLITHS	Oto. Code	MUSCLE	Muscle site (A,B,T,H)
B12346	YFT	80	UF	F	Y	2G	K	B
							P	B
B12346	WAH	80	UF	F	Y	2G	K	B

Spine

Record which spine or ray is collected. If you collect the first dorsal spine, write "D1". For marlins, if you collected other dorsal spines, note next to it the second dorsal spine "D2", the third dorsal spine "D3", the fourth dorsal spine "D4". For swordfish, if you collected the anal rays, note "A2".

Stomach

Record "Y" for yes, if you collected the stomach of the fish and "N" if you did not collect it.

Liver

Record "Y" for yes, if you collected a section of the liver of the fish and "N" if you did not collect it.

Gonads

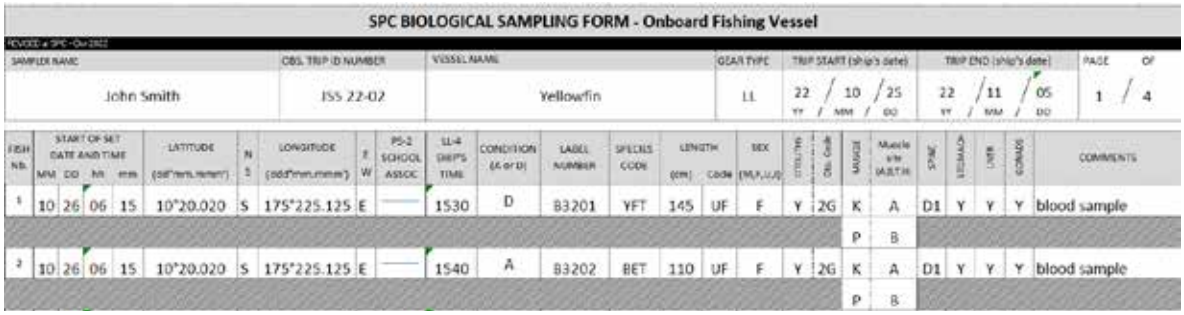
Record "Y" for yes, if you collected the gonads. If you collected only a single gonad, note "single gonad" in the comments section. If the gonad(s) is/are broken, note "broken gonad(s)" in the comments section.

Comments

If all of 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. You can specify the blood sample, single gonad, broken gonads in the "Comments" column. There is no need to repeat the information already captured by the other fields.

General comments

Record general information on your sampling, any difficulties encountered during sampling, such as a loss of samples, a broken item, if the captain did not allow sampling a fish, or if a sample type requested by the programme could not be collected.



SPC BIOLOGICAL SAMPLING FORM - Onboard Fishing Vessel

SAMPLER NAME	OBS. TRIP ID NUMBER	VESSEL NAME	GEAR TYPE	TRIP START (ship's date)	TRIP END (ship's date)	PAGE OF
John Smith	ISS 22-02	Yellowfin	LL	22 / 10 / 25 (YY / MM / DD)	22 / 11 / 05 (YY / MM / DD)	1 / 4

FISH No.	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 SHIPS TIME	CONDITION (A or D)	LABEL NUMBER	SPECIES CODE	LENGTH (cm)	Code	SEX (M,F,I,U)	STOMACH	Obs. Code	GAUGE	Muscle site (A,B,T,H)	SPINE	STOMACH	LIVER	GONADS	COMMENTS
1	10	26	06	15	10°20.020	S	175°225.125	E		1530	D	B3201	YFT	145	UF	F	Y	2G	K	A	D1	Y	Y	Y	blood sample
																			P	B					
2	10	26	06	15	10°20.020	S	175°225.125	E		1540	A	B3202	BET	110	UF	F	Y	2G	K	A	D1	Y	Y	Y	blood sample
																			P	B					

In this section, for onboard longline vessel or artisanal fishing vessel, record the bait used onboard. Record the three FAO species codes. These codes are marked in the "longline terminal gear identification guide".

Onboard a longline vessel: Immediately after measuring the fish, record the label number on the LL4 and the biological sampling form. (See the example provided below.) On your biological sampling form, indicate the kind of samples you have collected. When there is time (preferably before the end of the haul), copy the LL-4 form catch details (i.e. the 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.

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

OBSERVER NAME	OBSERVER TRIP ID NUMBER	SET No.	PAGE OF
John Smith	JSS 22-02	1	1 15

VESSEL NAME	MEASURING DEVICE	CALIBRATE +/-	SHIP'S START OF SET DATE AND TIME YY	MM	DD	hh	mm	START OF HAUL DATE YY	MM	DD
Yellowfin	Calipers	0' mm	22	10	26	06	15	22	10	26

CATCH DETAILS

SHIP'S TIME	HOOK No.	SPECIES CODE	GEAR INTERACTION CODE	CONDITION CODE CAUGHT	CONDITION CODE DISCARD	LENGTH (cm)	LENGTH CODE	FATE CODE	SEX M, F, I, U	COMMENTS / SSI TREATMENT
1530	2	YFT	–	A1	–	145	UF	RGG	F	B3201
1540	8	BET	–	A2	–	110	UF	RGG	F	B3202

SPC BIOLOGICAL SAMPLING FORM - Onboard Fishing Vessel

SAMPLER NAME	OBS. TRIP ID NUMBER	VESSEL NAME	GEAR TYPE	TRIP START (ship's date)	TRIP END (ship's date)	PAGE OF
John Smith	ISS 22-02	Yellowfin	LL	22 / 10 / 25 (YY / MM / DD)	22 / 11 / 05 (YY / MM / DD)	1 / 4

FISH No.	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 SHIPS TIME	CONDITION (A or D)	LABEL NUMBER	SPECIES CODE	LENGTH (cm)	Code	SEX (M,F,I,U)	STOMACH	Obs. Code	GAUGE	Muscle site (A,B,T,H)	SPINE	STOMACH	LIVER	GONADS	COMMENTS
1	10	26	06	15	10°20.020	S	175°225.125	E		1530	D	B3201	YFT	145	UF	F	Y	2G	K	A	D1	Y	Y	Y	blood sample
																			P	B					
2	10	26	06	15	10°20.020	S	175°225.125	E		1540	A	B3202	BET	110	UF	F	Y	2G	K	A	D1	Y	Y	Y	blood sample
																			P	B					
3	10	26	06	15	10°20.020	S	175°225.125	E		1600	A	B3203	SKJ	45	UF	U	Y	H	K	B	D1	Y	Y	Y	blood sample
																			P	B					
4	10	26	06	15	10°20.020	S	175°225.125	E		1700	D	B3204	WAH	98	UF	F	Y	2G	K	B	N	Y	Y	Y	broken gonads
5	10	26	06	15	10°20.020	S	175°225.125	E		1830	A	B3205	MLS	220	UFL	M	Y	H	K	B	D1	Y	Y	Y	D2, D3, D4 200 OFL
6																									

Figure 38. Filling out the biological sampling form from the LL-4 form information.

SPC BIOLOGICAL SAMPLING FORM – Port Sampling

In addition to the fields recorded in the biological sampling form used at sea, the port sampling form includes the fields below.

Port name

Record the name of the port in which the sampling occurs.

Sampling date

Record the date of the sampling.

SPC BIOLOGICAL SAMPLING FORM - Port Sampling								
Revised at SPC Oct2022								
PORT NAME: Nouméa	SAMPLER NAME: John Smith			SAMPLING DATE:	YY 22	MM 12	DD 12	
VESSEL NAME: Yellowfin				GEAR TYPE: LL				
TRIP START (Ship's date):	YY 22	MM 11	DD 29	TRIP END (Ship's date):	YY 22	MM 12	DD 11	

Date of the first successful set

Record the date when the fishing vessel deployed a set and caught fish. Do not take into account the date of "Transit" or "A day at sea but not fishing" or the date where the fishing vessel deployed a set and did not catch any fish (especially onboard purse seiners).

Date of the last successful set

Record the date when the fishing vessel deployed, its last set and the fish caught before returning to port. Do not take into account the date of "Transit" or "A day at sea but not fishing" or the date where the fishing vessel deployed a set and did not catch any fish (especially onboard purse seiners).

If the sampled fish are coming from a well with only one set, do not fill this field and place a dash.

Fishing area

Latitude and longitude positions can be recorded from the GPS of the vessel. Record the latitude (dd°mm.mmm) and the longitude (ddd°mm.mmm) to three decimal minutes.

"From latitude", record the southernmost successful set position. "To latitude", record the northernmost successful set position, and do not forget to fill in whether this position refers to north (N) or south (S) of the equator.

"From longitude", record the westernmost successful set position. "To longitude", record the easternmost successful set position and do not forget to fill in whether this position refers to east (E) or west (W) of the equator.

FIRST SUCCESSFUL SET (Ship's date):		YY 22	MM 11	DD 30		LAST SUCCESSFUL SET (Ship's date):	YY 22	MM 12	DD 10	
FISHING AREA	From Latitude (dd°mm.mmm')	19°20.000 S			N / S	To Latitude (dd°mm.mmm')	23°20.000 S			N / S
	From Longitude (ddd°mm.mmm')	165°15.000 E			E / W	To Longitude (ddd°mm.mmm')	164°10.000 E			E / W

If the sampled fish are coming from a well with only one set, do not fill the field "To latitude" and "To longitude". Place a dash in these two fields.

FISHING AREA	From Latitude (dd°mm.mmm')	10°20.000 S	N / S	To Latitude (dd°mm.mmm')		N / S
	From Longitude (ddd°mm.mmm')	175°15.000 E	E / W	To Longitude (ddd°mm.mmm')		E / W

On a chart when facing the 180°meridian, the lines of longitude West are on the right side of the chart and the lines of longitude East are on the left side of the chart.

On the left of the 180°meridian, the number of degrees East increase as you move towards the East. On the right of the 180°meridian, the number of degrees West decrease as you move towards the West.

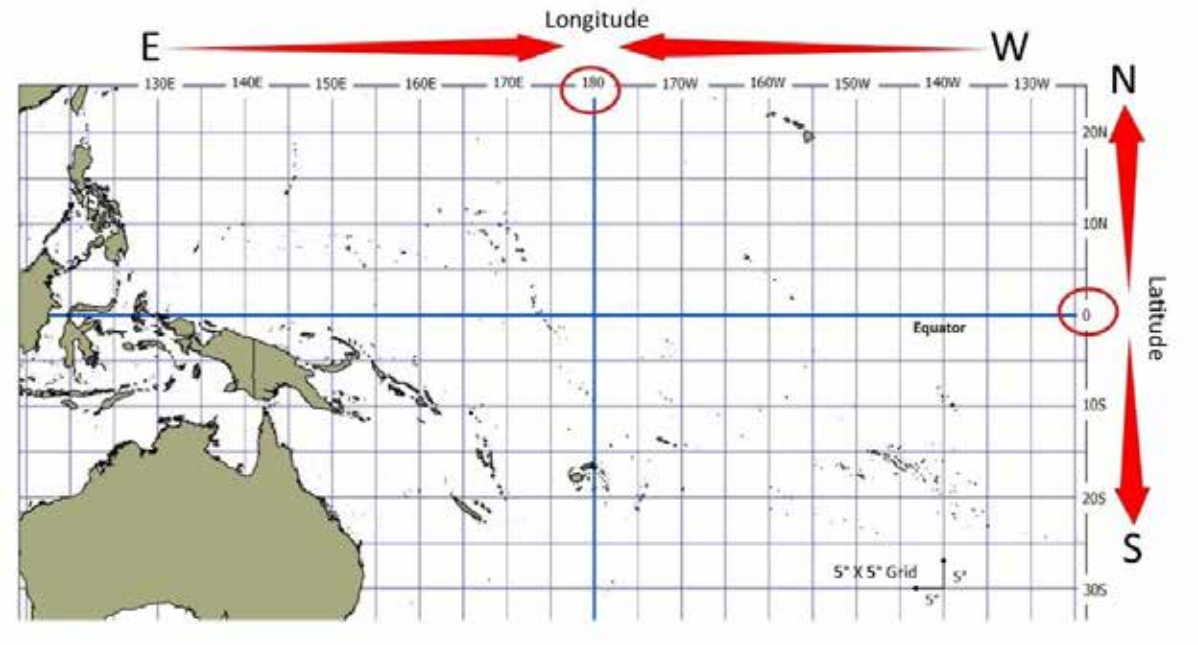


Figure 39. Map of the Pacific Ocean with latitude and longitude details.

When selecting the latitude and longitude for a fishing area, verify in which hemisphere the sets were done.

For latitude

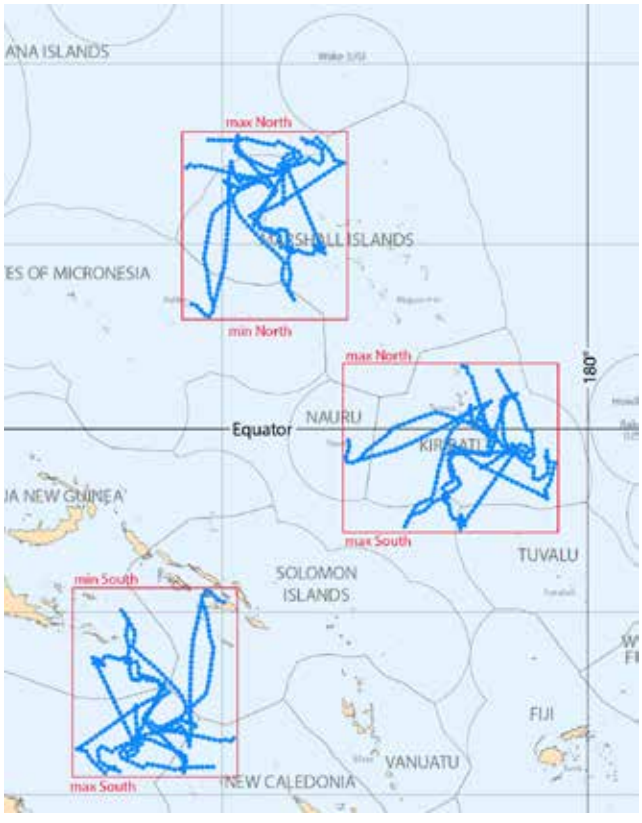


Figure 40. Example of sets deployed only in the latitude North, across the equator line and only in the latitude South.

If all of the sets were deployed on the latitude North OR on the latitude South, note the highest number in North degree value and the lowest number in North degree value, or note the highest number in South degree value and the lowest number in South degree value.

If all of the sets were deployed on the latitude North AND the latitude South, note the highest number in North degree value and the highest number in South degree value.

For longitude

If all of the sets were deployed on the latitude East OR on the latitude West, note the highest number in East degree value and the lowest number in East degree value, or note the highest number in West degree value and the lowest number in West degree value.

If all of the sets were deployed on the longitude East AND the longitude West, note the lowest number in East degree value and the lowest number in West degree value.

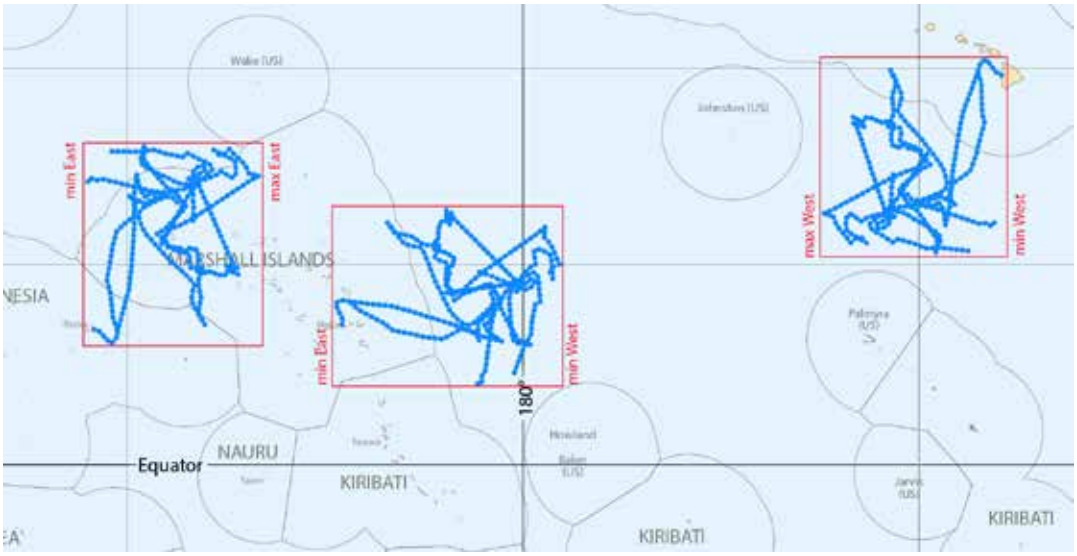


Figure 41. Example of sets deployed only in the longitude East, across the 180 degree line and only in the longitude West.

Sampled well number

Record the well number from which the sampled fish was selected. Note "Unknown" if you could not identify the well. From purse seiner, collect fish preferably from a well containing only one set.

Refrigeration method onboard fishing vessel

Record the refrigeration method onboard the vessel from which the sampled fish originate. Refrigeration method could be: blast freezer; ice; brine well; brine spray; chilled sea water; refrigerated sea water; or no refrigeration method.

Was bait used?

Circle Yes or No. Record the three FAO species code. Refer to the manual and guide for FAO species codes and the "Longline terminal gear identification guide". You can obtain assistance from fisheries observers or the coordinator if you cannot obtain the information at the port.

Sampled well number:	Unknown	Refrigeration method onboard fishing vessel:	Ice	Was bait used during the fishing trip? If yes, species ID: CHP	(Yes) / No

Fish state

Record the fish state when you start sampling the fish. Note "FH" if the fish is fresh, "FZ" if the fish is frozen, "SD" if the fish is slightly defrosted or "D" if the fish is defrosted. A slightly defrosted fish is a fish with the skin completely defrosted and the first few 5 mm of the muscle soft. A defrosted fish will have the muscle soft and, when pressing with the hand, a change of width.

Gonads

Record the preservation method of the gonads. Gonads in port can be placed in formalin. Record "F" if the gonads are collected and preserved frozen, record "V" if the gonads are collected and preserved in a vial with 10% formalin, record "C" if the gonads are collected and preserved in a

cassette with 10% formalin, record "FV" if the gonads are collected, preserved frozen and in a vial with 10% formalin, record "FC" if the gonads are collected, preserved frozen and in a cassette in 10% formalin. Record "N" if the gonads are not collected.

Comments

Record the weight of the gonads (weighing only the entire and complete gonads) as well as any other relevant sampling information (e.g. additional samples and weight of the gonads).

SPC BIOLOGICAL SAMPLING FORM - Port Sampling

Revised at SPC-Oct2022

PORT NAME: Nouméa			SAMPLER NAME: John Smith				SAMPLING DATE:	YY 22	MM 12	DD 12										
VESSEL NAME: Yellowfin							GEAR TYPE: PS													
TRIP START (Ship's date):	YY 22	MM 11	DD 29				TRIP END (Ship's date):	YY 22	MM 12	DD 11										
FIRST SUCCESSFUL SET (Ship's date):	YY 22	MM 11	DD 30				LAST SUCCESSFUL SET (Ship's date):	YY 22	MM 12	DD 10										
FISHING AREA	From Latitude (dd°mm.mmm')	19°20.000 S	N / S				To Latitude (dd°mm.mmm')	23°20.000 S	N / S											
	From Longitude (ddd°mm.mmm')	165°15.000 E	E / W				To Longitude (ddd°mm.mmm')	164°10.000 E	E / W											
Sampled well number: P3			Refrigeration method onboard fishing vessel: Brine				Was bait used during the fishing trip? Yes / No If yes, species ID:													

FISH Nb.	LABEL NUMBER	SPECIES CODE	LENGTH cm	LENGTH code	SEX (F,M, U,I)	FISH state	OTOLITHS	OTO. Code	SPINE	MUSCLE	Muscle Site (A,B,T,H)	STOMACH	LIVER	GONADS	COMMENTS
1	P12345	YFT	100	UF	M	FZ	Y	2G	D1	K	B	Y	Y	FV	
										P	B				
2	P12346	DOL	90	UF	F	FZ	Y	H	N	K	B	Y	Y	FC	
										P	B				

Figure 42. Example of a biological sampling form – port sampling.

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Packaging the samples

ONBOARD OR AT PORT

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. If you have sampled blood, you do not need to roll the blood sample with the other samples. If you have collected muscle with a biopsy punch tool or a widget gene tagging tool, wrap each vial lid with a 1 cm wide strip of parafilm tool, and place the sample in a dedicated box. If you preserved the gonads in 10% formalin, place them in a dedicated sealed plastic box.

When you have collected the samples, **put them in the freezer as soon as possible (except for the otoliths)**. For example, during sampling, place the samples in the shade in a bucket of ice or in a cooler. Do not leave the samples in the heat. When taking genetic samples, keep filled vials in the shade and cool while on deck, as with other tissue types. Keep the genetic storage box in the freezer at all times, when not actively loading samples or transporting between freezers. As soon as possible, place vials in the frozen storage box.

Ensure all of your frozen samples placed in the plastic bags 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** or your **port name, the fishing vessel** and the **sampling date**. Ensure, when storing the samples in a freezer onboard, that the bag is either labelled and the crew (especially the cook) knows about it so the samples are not thrown away or used for cooking.

Place the vials containing the otoliths in a dry re-sealable bag in a safe place. Use a permanent pen to write on the bag your **trip ID number** and the **disembarking port name** or your **port name, the fishing vessel** and the **sampling date**. Do not freeze them. Be aware, if you fly with the otolith vials, that the caps of the vials need to be sealed with sticky tape or electric tape. This will ensure that the lid does not pop up with the pressure variation in the aeroplane.

Label the designated genetic samples storage box with trip ID number and disembarking port name, or port name/fishing vessel/sample date. Write the label on masking tape and press it onto the box lid (as writing directly on the lid will eventually wipe off). Keep the storage box in a freezer onshore.

After sampling at sea or at port

When you return to the port or to the fisheries offices, hand over the samples and the data to the observer coordinator or the debriefer. If not, scan the forms and send them to SPC. During the pre-debriefing, let the debriefer or SPC know of any difficulties you may have had with the sampling procedures.

You must ensure that each genetic vial lid is wrapped with a 1 cm wide strip of parafilm to avoid spills during shipment, and that the genetic box is labelled.

NB: samples from multiple trips can be consolidated into a single storage box for shipment, as long as the labels for all trips are noted on the lid.

You must ensure that all of the samples are stored in a freezer in a labelled bag (except for the otolith's bag). 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 SPC 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 SPC as well as the local fisheries authority.

Verify that each container with 10% formalin is labelled with the sampling date and vessel name on the container cap. Gather all of the containers in a store locked up and in a well-ventilated place. Do not forget to remove the 10% formalin after 14 days. (See the gonads sampling at the port.)

Notes

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