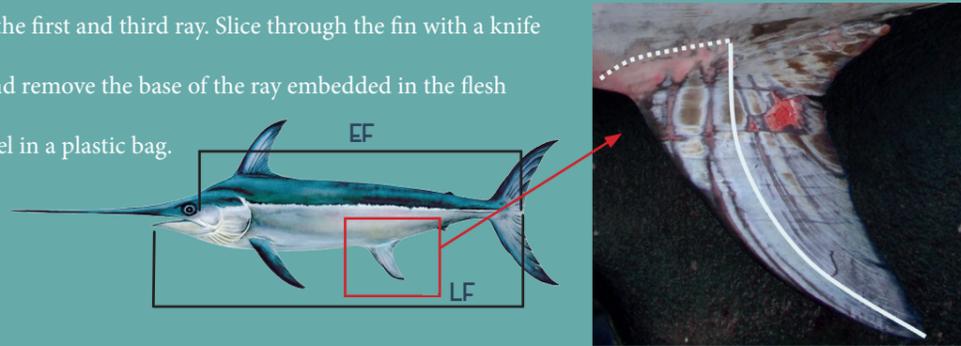


BIOLOGICAL SAMPLING PAMPHLET

HOW TO COLLECT ANAL FIN RAYS FROM SWORDFISH

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



WHAT TO DO AFTER SAMPLING A FISH?

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

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



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

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

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

Example of Sampling Protocol page:

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

WHAT TO DO BEFORE ARRIVAL TO PORT ?

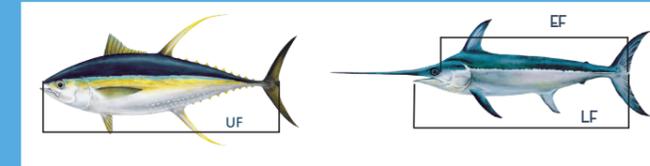
- Before arriving at port, gather all of your frozen samples in a large plastic bag and label the bag with your ID number and the disembarking country name. Use a permanent pen to write the information on the large plastic bag.
- When the fishing vessel is 3/4 full, contact **Caroline Sanchez**: carolines@spc.int/+687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

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



HOW TO MEASURE THE FISH

- Measure the fish: UF for tuna species, and both LF and EF for swordfish. 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.
- For tuna species make sure that you only sample 5 fish for the size range concerned.



HOW TO USE THE CABLE TIE LABELS



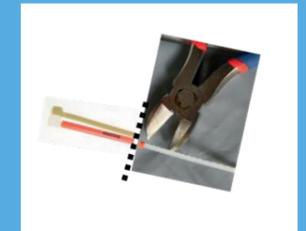
- Place the cable tie with 5 labels through the mouth of the fish.



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



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



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

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

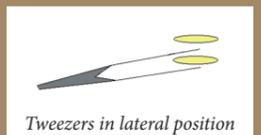
HOW TO SAMPLE BLOOD

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



HOW TO EXTRACT THE OTOLITHS FROM TUNA

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



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

Remove the top of the head

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



Cut the otic capsule with nail removers and side cutters

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



Drill cores under the gills

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



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



FIRST DORSAL SPINE SAMPLING

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



HOW TO COLLECT OTHER INTERNAL BIOLOGICAL SAMPLES

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



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



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



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



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



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



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



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



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

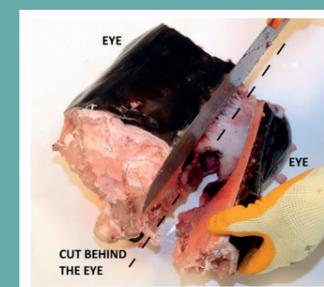
! When you are sampling several fish, do not leave the samples under the sun. Place all samples immediately in an esky, a bucket of ice, or directly in the freezer.

HOW TO SAMPLE A SWORDFISH HEAD

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



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



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



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