

DO NOT FLY WITH FORMALIN

**NOAA Fisheries Pelagic Observer Program
Gulf of Mexico/Atlantic Bluefin Tuna Sampling Protocol
January 2015**

NOAA Fisheries Service, Southeast Fisheries Science Center
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Sampling Labeling

All samples taken should be clearly labeled so that the samples can be referenced back to your data forms. All bluefin brought up on board and either kept and/or sampled and then discarded will be assigned a sequential alphanumeric on your animal logs' "Carcass Tag" data field. These numbers will be BFT01, BFT02, etc.

These sample numbers are intra-trip specific; you will not continue to assign the numbers sequentially on subsequent trips, rather on each new trip you will begin back at BFT01.

On ALL BFT samples collected, you will clearly label the samples with your TRIP IDENTIFICATION # **AND** the CARCASS TAG # **AND** the DATE collected.

For example: An observer on trip A03002 collecting an otolith from the vessel's first bluefin tuna of the trip on May 15th, 2007 will label the otolith:

A03002	BFT01	5/15/07
Trip Identification #	Carcass Tag #	Date of Capture

Because sample labeling can become smudged or fade, samples should be double-labeled; e.g. for otoliths the appropriate OTOLITH LABEL (provided on waterproof paper) needs to be completed with a pencil and inserted into the vial; or for other hard part and soft tissue samples be sure the appropriate SAMPLE LABEL (provided on waterproof paper) needs to be completed with a pencil and inserted into the zip-lock bag, the Trip Identification #, Carcass Tag #, and Date of Capture needs to also be written on the zip-lock with a black sharpie.

Note: Federal HMS regulations require fishermen to "provide access" to "any.....space used to hold, process, weigh, or store fish".

They do not require fishermen to process/dress tuna in the manner most likely to yield the samples we want, nor do they require that the fishermen allow the observer to cut and handle the fish themselves, however, regulations that have recently become effective **do** require that fishermen lift on board the vessel a 700 lb fish that they do not intend to keep solely for the purpose of observer sampling, **when practical**.

You may encounter variations in dressing methods between vessels, so you may be limited at what you are able to collect on the vessel; however you might have another chance to sample the fish later on during the weigh-out at the fish house.

Sampling Procedures

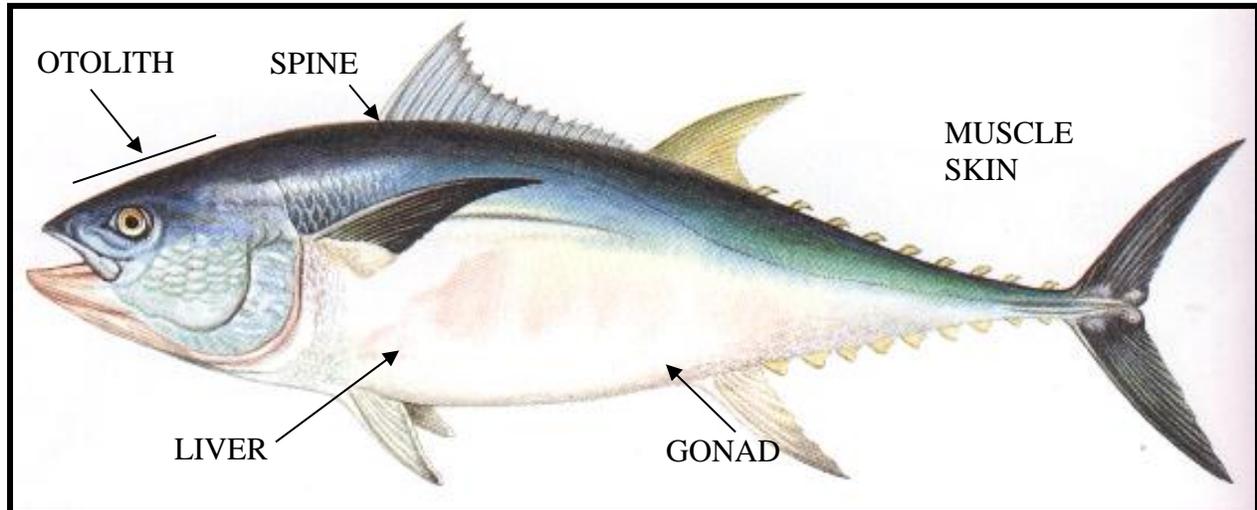


Figure 1. Specific parts of the Bluefin tuna for sampling.

Sampling for hard parts

Detailed sampling for hard parts' procedures and pictures were obtained from the ICCAT On-Line Manual, http://www.iccat.es/pubs_FieldManual.htm.

These procedures are only a guideline. We realize you may encounter difficulties extracting samples from tuna at-sea, and sampling may have to wait until the weigh-out at the fish house. Please appropriately label the bluefin tuna with the trip identification #, carcass tag #, and date.

A wide range of hard parts have been used to age tuna and billfish species. These include otoliths (sagittae, e.g. Atlantic bluefin tuna), the 1st dorsal fin spine/ray (where spines are generally hard and rays soft) or anal fin spines/rays (e.g. swordfish (spines), yellowfin tuna, bigeye tuna (rays)), vertebrae (generally from the caudal peduncle, vertebra no. 35, e.g. Atlantic bluefin tuna, bigeye tuna).

1. Otolith Removal

Assuming the head is cut off on the vessel approximately at the location needed for otolith extraction, take the head from the butcher before it is discarded overboard.

Otolith sampling

Sagittal otoliths are small (between 7 and 20 mm in size approximately) calcified structures found in the semicircular cavities of the inner ear, situated at the base of the brain. The sagittal otolith is the largest of the three otoliths found in each inner ear of the bluefin tuna.

There are two main techniques of removal: transverse head section and frontal head section. In the second, a frontal section of the superior part of the cranium is made, passing above the eye and parallel to the major axis of the fish. Only, the transverse technique is detailed here.

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Transverse head section:

- a. Trace an imaginary line perpendicular to the horizontal fish, which passes through the mid-point between the posterior end of eye and the pre-operculum (**Figure 2A**). A ruler is recommended for dividing this distance in two.

Additional
Notes:

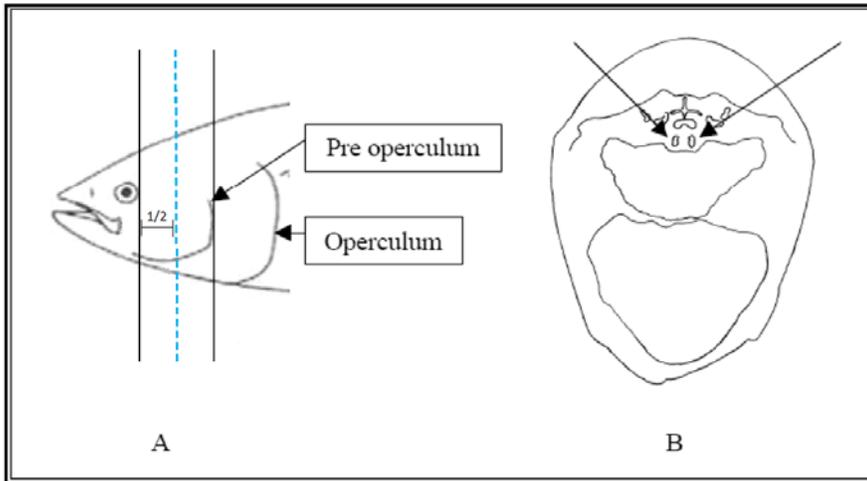


Figure 2.

A. Tracing the imaginary line (dotted) along which to make the cut.

B. View of the cavities where the pair of otoliths is found in the back of the head.

- b. Once this point is marked, make a cut in the top part or back of the head at the level of the imaginary line (**Figure 2A**).
- c. Use a metal saw and cut down through the head perpendicular to the horizontal axis of the fish (**Figure 2B**). The sectioned part of the head contains the otoliths. The cavities below the brain in the upper part of the head should be searched to find the otoliths. If they are not found here, it may be that they are in the other part of the sectioned fish.
- d. Using fine forceps and with great delicacy to avoid breaking these fragile pieces, extract each otolith. Both otoliths must be collected from each specimen. If the otolith has broken, try to recover the pieces and keep them all together.
- e. The otoliths must then be removed from the very fine transparent capsule. Once extracted, rinse them in water and pat them dry with a clean cloth/paper towel.
- f. Place the dried otoliths along with the OTOLITH label (use a pencil to fill-in the label) in the provided vial. Make sure the correct Trip Identification #, Carcass Tag #, and date is visible. Then place this vial in a quart zip-lock freezer bag. Write at least the Trip Identification #, Carcass Tag #, and 'OTOLITH' on the vial and on the zip-lock bag with a black sharpie.
- g. Please do not store the otoliths in a refrigerator or freezer, cold temperatures will increase the potential for breaking.
- h. If you are unable to extract the otoliths and have to send part of the tuna's head to our facility, then please indicate this on the Biological Sampling Check-list.

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- i. Place the head parts along with the OTOLITH label (use a pencil to fill-in the label) in a 1-gallon zip-lock freezer bag. Make sure the correct Trip Identification #, Carcass Tag #, and date is visible. Write at least the Trip Identification #, Carcass Tag #, and 'OTOLITH' on the zip-lock bag with a black sharpie.
- j. If the head parts are removed at-sea, then it needs to be kept frozen or on ice.

2. Dorsal fin spine/ray

At this point we are not sure if the vessels and/or fish house personnel will allow us to remove the first dorsal fin spine. However, in the event we gain clearance (and this will more likely be from the fish dealers rather than the vessel operators), follow the procedure below.

- a. The first spine of the first dorsal fin should be collected. The spine must be pulled out whole from the base.
- b. Using a knife, cut the membrane joining the 1st and 2nd dorsal fin rays (**Figure 3**). Push the spine forward progressively until the ligament breaks. Twist the spine left and right alternatively until it comes loose and pull to finally extract it (**Figure 4**).

Figure 3. Insert the knife into the membrane separating the first two spines of the 1st dorsal fin.

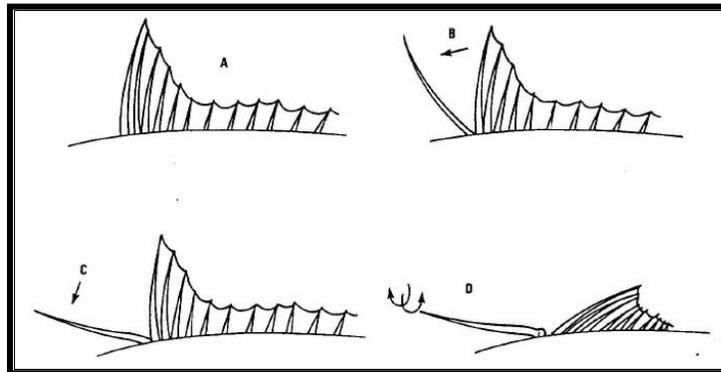
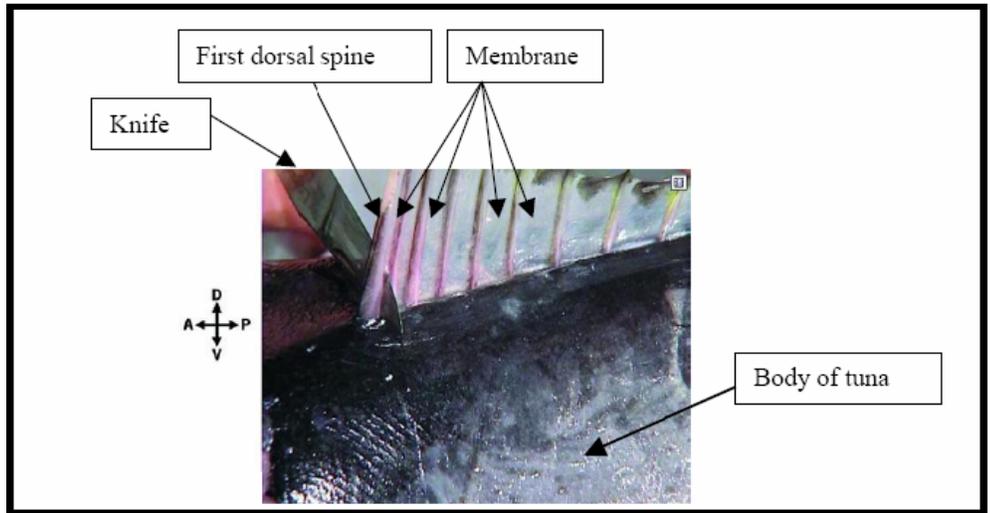


Figure 4. Technique of extraction of the first spine of the bluefin tuna dorsal fin.

- c. Spines should be preserved in a quart zip-lock freezer bag, which should be kept in a cool place (refrigerated or frozen). Remembering that the piece forming the base of the spine is the most important since it is the part used for age interpretation.

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- d. Place the spine and SPINE label (use a pencil to fill-in the label) in the provided quart zip-lock freezer bag. Make sure the correct Trip Identification #, Carcass Tag #, and date is visible. Write at least the Trip Identification #, Carcass Tag #, and tissue type 'SPINE' on the zip-lock bag with a black sharpie.
- e. Spines should be stored frozen or kept on ice.

Sampling for soft tissues

A variety of soft tissues (red and white muscle, skin, organs (respiratory, circulatory, digestion, reproductive, etc.)) can provide vital information on the health, genetic composition, and the maturity level of bluefin tuna.

If the tail section of the fish is cut off at sea, take the piece from the butcher before it is discarded overboard and remove the upper and lower lobes of the caudal fin if possible. In the event the tail section is *not* removed on the vessel, your ability to collect the sample will have to be discussed with the fish house personnel during the weigh-out back at the dock.

Important note: Federal regulations require that the tail sections of tuna must remain on the fish until offloading, although many vessels still cut off the tail. However, it is extremely important that an **observer not ask fishermen to cut off the tail for sampling purposes until after the unloading takes place.** It should be understood that if the vessel decides to remove the tail at sea, that is solely the decision on the Captain/crew and not in any way related to the observer's sampling requirements.

1. Muscle & Skin

- a. Obtain a piece of flesh and separate the outer skin layer with the inner muscle layer with your knife (**Figure 6**). Obtain one cube of muscle, at least 2-inch x 2-inch x 2-inch in size, and a 2-inch length of skin. Be very mindful of cutting on a fish that the vessel intends to land and ask permission before taking any flesh. This may be best done at the dock as they trim pieces off.

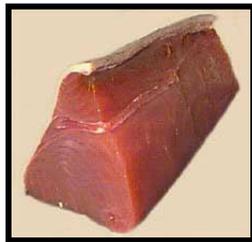


Figure 6. Use a knife to separate the muscle from the skin. **Muscle and skin samples are best taken at the dock.**

- b. Please place the muscle along with the MUSCLE label (use a pencil to fill-in the label), in the provided quart zip-lock freezer bag. Make sure the correct Trip Identification #, Carcass Tag #, and date is visible. Write at least the Trip Identification #, Carcass Tag #, and tissue type 'MUSCLE' on the zip-lock bag with a black sharpie.

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- c. Please place the skin along with the SKIN label (use a pencil to fill-in the label) in the provided quart zip-lock freezer bag. Make sure the correct Trip Identification #, Carcass Tag #, and date is visible. Write at least the Trip Identification #, Carcass Tag #, and tissue type 'SKIN' on the zip-lock bag with a black sharpie.
- d. The muscle and skin samples should be stored frozen or kept on ice.

The following two tissue types must be obtained before the fish is completely gutted.

2. Liver

- a. The liver is a source of fat and is a single lobe organ found in the anterior portion of the fish, normally located within the body cavity below the pectoral fin. Livers can range in color from light to darkish red or brown to a deep purple (**Figure 7**).

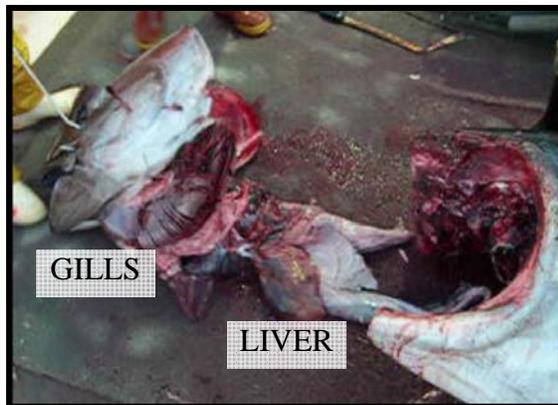


Figure 7. Bluefin tuna with head removed and internal organs displayed.

- b. Remove at least 2-inch cube of liver.
- c. From the above main piece, cut one small .5 cm³ piece (about the size of a pencil's eraser) and place the sample along with LIVER SUBSAMPLE label in the separate provided bottle. Bottles are filled with 10% neutral buffered formalin (~10 ml); therefore, wear the provided Nitrile gloves and safety glasses when putting samples in bottles. Put the bottle back in the quart zip-lock freezer bag it was initially stored in, and make sure the outside of the zip-lock bag is identified with the Trip Identification # and Carcass Tag # with a black sharpie.
- d. Please place the main liver piece along with the LIVER label (use a pencil to fill-in the label) in the provided quart zip-lock freezer bag. Make sure the correct Trip Identification #, Carcass Tag #, and date is visible. Write at least the Trip Identification #, Carcass Tag #, and tissue type 'LIVER' on the zip-lock bag with a black sharpie.
- e. The main liver piece should be stored frozen or kept on ice, the subsample in formalin needs to be kept at room temperature and should be stored in the provided screw-top bucket.

3. Reproductive Tissue (ovaries and testes)

Please try to remove the reproductive tissue as soon as possible after capture to avoid gonad decomposition.

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The gonad is the only bi-lobed organ in the abdominal cavity dorsal to the anus, and will be attached to the upper-rear abdominal wall. Depending on the stage of reproductive development the ovaries of females will appear as elongated lobes which may be orange, yellow or pinkish-red in color. The testes appear as triangular lobes that are usually whitish in color.

Gonad removal via the head

In many cases, once the head has been removed, the fish will be gutted via the head opening.

(Figure 8).



Figure 8. Once the head is removed, gonads can be removed through the head opening.

- a. Once the fish has been gutted, locate the bi-lobed gonad.
- b. Make sure to collect the entire gonad trying not to cut either of the lobes.

Gonad removal via the abdomen

- a. Use a sharp knife and insert its tip just inside the anus. Make a shallow cut through the ventral abdomen up to the base of the pelvic fin; pull the blade out away from the abdomen as you cut so that the knife is less likely to slice into the gonad.
- b. Grab the two lobes and carefully pull them away from the abdominal wall. Cut the posterior end from the abdominal wall without cutting either of the lobes.

Gonad weighing instructions

- c. The weight of the whole gonad must be recorded. Please remove any excess attached connective tissue and attached fat prior to weighing.
- d. Place the gonad in a 2-gallon zip-lock freezer bag (**Figure 9**). Make sure the Pesola scale is zeroed (if not, zero the scale by turning the screw at the top).

Use the hook on the scale and make a puncture near the top of the 2-gallon zip-lock freezer bag and record gonad weight (to the

Figure 9. Weigh gonad in 2-gallon zip-lock freezer bag to nearest decimal. Large gonads may require sectioning to determine whole gonad weight.



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nearest tenth of a decimal, e.g. 6.7 kg). Gonads of large bluefin tuna may weigh over 10 kg, therefore, the gonad may need to be weighted one lobe at a time or sectioned and weights summed to determine whole gonad weight. It is important to get an accurate gonad weight, if the gonad is too heavy please section and sum weights.

Record the whole gonad weight and sex on the BLUEFIN TUNA SAMPLING CHECKLIST. Once the whole weight of the gonad is determined, the 2-gallon freezer zip-lock bag can be discarded.

- e. Once the whole gonad weight is recorded, locate the center of either lobe of the gonad (**Figure 10**).



Figure 10. Testes and ovaries with central gonad region circled. **Three** gonad subsamples should be taken from the center of either lobe.

- f. Using the corer, remove **three** samples of gonad tissue about the size of a sugar cube (**Figure 11**). Please place each sample along with GONAD SUBSAMPLE 1, GONAD SUBSAMPLE 2 or GONAD SUBSAMPLE 3 labels in each of the separate provided bottles. Bottles are filled with 10% neutral buffered formalin (~10 mL); therefore, wear the provided Nitrile gloves and safety glasses when putting samples in bottles. Put the three bottles back in the quart zip-lock freezer bag they were initially stored in. Label the GONAD BAG label (use a pencil to fill-in the label) with the Trip Identification # and Carcass Tag # and place in quart zip-lock freezer bag. Make sure the outside of the zip-lock bag is identified with the Trip Identification #, Carcass Tag #, and tissue type 'GONAD' with a black sharpie.



Figure 11. Use corer to remove three samples from the central gonad region. Place corer on the exterior of the gonad and twist corer until a sample the size of a sugar cube is removed. Place each sample in a separate bottle, make sure the cap is secured tightly, and store bottles in labeled zip-lock bag.

- g. The formalin gonad samples should be stored in the provided screw-top bucket. Gonad samples in formalin should **not** be frozen.
- h. At the end of each trip, place all formalin samples collected from that trip in a 1-gallon zip-lock freezer bag labeled with the Trip Identification # and dates of the trip.

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Personnel Notification: Panama City, FL, all BFT samples are to be sent to the NMFS lab in Panama City, attn: Ashley Pacicco. Please use the pre-printed Panama City labels. Call AND email Ashley at the number/ email address listed below to notify her you are sending samples; please include tracking number from the package(s).

After recording all information on the BFT Sampling Checklist, make **2** copies of each BFT Sampling Checklist. Send one copy in with your other paperwork/data to the Miami Lab POP; the **original checklist should remain with the frozen samples**. Keep one copy for yourself and have it available during debriefing.

Panama City NMFS Lab sample contact:

Ashley Pacicco
NOAA Fisheries Service
3500 Delwood Beach Road
Panama City, FL 32408
Phone: 850-234-6541 (ext. 240)
Email: ashley.pacicco@noaa.gov

Appendix: different vials in use.

Note that there are at least four separate types of pre-filled formalin vials that you may be provided with in order to preserve samples. These vials may be of different shapes, colors, and approximate sizes (**Figure 12**); do not confuse them. All vials containing formalin, should be marked, at a minimum: “10 % Formalin, poison (or international poison sign)”.



Figure 12. Examples of the various Formalin vials used.

*Place all samples in their respective zip-lock bags (otolith with head, spine, muscle, skin, and liver) in the provided 1-gallon zip-lock freezer bag and label with the Trip Identification #, Carcass Tag #, and date of capture. Make sure these samples are kept frozen at all times.
*Otoliths must be kept dry but do not freeze.
*Gonad and liver samples in formalin should not be frozen.