



Food and Agriculture
Organization of the
United Nations



Indian Ocean Tuna Commission
Commission des Thons de l'Océan Indien

Collecting, preserving, storing and recording samples

IOTC ROS SFO TR11.5

Category: Sampling procedures

[IOTC ROS SFO TR11]



CapMarine
Capricorn Marine Environmental

Part of a Scientific Observer's functions, together with recording biometric data, is to collect samples for further analysis ashore. In most cases this requirement will be independently specified for selected trips and species. Sampling commonly includes:

- collecting otoliths from selected species for age and growth studies, this can extend to spines and vertebra;
- collecting stomach samples for study on diet;
- collecting gonads for histological studies on maturity; and
- collecting tissue samples for DNA analysis for accurate species identification.

All these sampling requirements have their own procedures for collection, storage and labelling.



Sample Collection

Basic tools required for sample collection

Dissection Kit sufficient to collect most samples such as

- Dissecting gonads to determine maturity
- Opening stomach to view contents for diet related studies
- Collecting Tissue samples for DNA

A good dissection kit should contain

- Forceps (various sizes including plastic forceps)
- Scalpel & scalpel blades
- Dissection scissors
- Stainless steel side cutters
- Waterproof paper and pencil
- Ziplock plastic bags & waterproof marker for labeling



A range of basic or genetic tools are required to collect most samples that will be asked for and observers should always carry these with them. [Often called a “dissection kit”] These would include items such as

- Forceps (various sizes including plastic forceps)
- Scalpel & scalpel blades
- Small robust knife
- Stainless steel side cutters
- Waterproof paper
- Pencils for labelling waterproof paper
- Ziplock plastic bags (various sizes)
- Waterproof marker for labelling plastic bags

A good dissection kit should be sufficient to collect most biological samples such as

- Dissecting gonads to determine maturity
- Opening stomach to view contents for diet related studies
- Collecting Tissue samples for DNA

Where there are specific requirements such as collecting otoliths from tuna or swordfish then more specialised equipment may be necessary. i.e. fine toothed saw to cut sections off a tuna head to access the otoliths.



Sample Collection

Knowledge of the fish internal anatomy

A basic knowledge of fish internal anatomy is required to identify

- Stomach
- Intestine
- Liver spleen
- gonads



Knowledge of the fish internal anatomy

Observers should have a basic knowledge to identify both a fish's external and internal anatomy. To collect most biological samples then must be able to identify:

- Stomach;
- Intestine;
- liver and spleen; and
- gonads.

The stomach opening starts from directly behind the gill cavity and is robust and muscular, (on some tuna longline vessels these are retained and frozen). The stomach is also surrounded by the liver and spleen and then opens into the intestine that is relatively short in tuna and leads to the cloaca just anterior to the anal fin.

The gonads are found close to the base of the stomach cavity close to the backbone and they are also connected to the cloaca with the intestine. (*remember fish spawn by releasing their eggs into the water through the cloaca*) It is important not to confuse the gonads with the intestines, especially in smaller and immature fish.

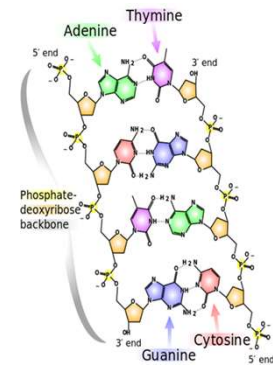


Sample Collection

Collecting genetic or DNA samples

The following tissues are suitable for DNA sampling from fishes

- musculature
- gill tissue
- eye
- whole specimen
- fin clips



There has been an increasing requirement for genetic samples to be collected to look at managing isolated stocks. Detailed instructions on which part of the body to sample (fin clip, muscle, or liver), how many samples required per site, and storage methods (90% alcohol or frozen) should be provided by the scientist requesting the samples.

There are three main factors to note when taking DNA samples:

- where and what to sample;
- how to take the sample (standard operating procedure [SOP]); and
- what tools do you require?

The most common parts to collect samples from a fish are:

- Musculature: Remove one or more cubes (5 – 7 mm) of lateral muscle from the right side of a whole specimen;
- Gill tissue: Remove one or more gill arches with attached filaments from the right side of the specimen;
- Eye: The right eye can be removed from small fish (if required);
- Whole specimen: If the fish is very small then the whole specimen can be frozen;
- Fin clips: Remove a part of the pelvic, pectoral, or caudal fin. This is preferred if the animal is still alive as it is a non-lethal form of sampling, such as for species of special interest.



Sample Collection

Items required for collecting genetic samples

- Dissection kit plus
- Eppendorf vials (various volumes, 15 to at least 50 ml)
- fixative solution [70 to 95% alcohol, Bouin's solution, or freeze]
- surgical gloves



SOP for collecting genetic samples

- photograph fish & record biometrics [length/weight/sex & maturity]
- disinfect instruments with alcohol
- take sample [approx. 1 cubic cm]
- place tissue in a vial [**preservative must be at least 3X volume of sample**]
- clearly label sample with all details



Have all the equipment on hand when taking samples, which should include:

- Dissection kit;
- Eppendorf vials (various volumes, 15 to at least 50 ml);
- fixative solution (these should be pre-mixed, and a range of solutions are available, 70 to 95% alcohol, Bouin's solution, alcoholic formalin etc.) or samples may be required to be frozen;
- surgical gloves are important to prevent contamination of the sample.

The general operating procedure for collecting genetic samples are:

- photo sampled fish;
- record biometrics of fish [length / weight / sex & maturity];
- use scalpel or scissors [disinfected] with alcohol];
- take small tissue or fin sample of approx. 1 cubic cm depending on what's requested;
- place tissue in a vial with fixative; and
- **clearly label sample**



Sample Collection

Extracting Otoliths

Collecting otoliths from tuna and swordfish the head is sawn open to expose the otolith cavity



Observers may be expected to collect otoliths for age and growth studies from specific species. This is not always that easy and is especially difficult for both tuna and swordfish as they are so small and delicate. As such often observer are required to only sample for the part of the head that contains the saccule with the otoliths.

The basic procedure is to open the skull of the fish (use knife or saw) to access cavities containing otoliths. This can be done in several ways:

- dorsal cross-section - most used, on all types of fish (any species, individual size or cranial morphology)
- transversal cross-section - performed by separating the body of the fish's head, may be more practical on certain species (i.e. swordfish, tuna)
- ventral cross-section - through the gills; does not damage the appearance of a fish.
- after cutting, remove the brain and tissue to access the semi-circular canals (saccule) containing the otoliths and extract the otoliths using tweezers
- clean the otoliths and store in clearly labelled paper envelopes.



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ANY QUESTIONS?



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