

PROTOCOL FOR COLLECTION OF SAMPLES FOR THE BYP

There are two different methods for the collection and storage of fish material in terms of the kind of biological analysis: FREEZING and ETHANOL. Freezing is the best method for the collection and storage of specimens for electrophoresis and other biological analyses, like biochemistry and physiology, because of the liability of enzymes in vitro. Proper cryogenic storage will preserve enzyme activity and minimize breakdown. Samples preserved on ethanol, **can only be used for genetic studies** such as DNA amplification and sequencing.

The general procedures for processing the samples are as follows:

A. CLEANLINESS OF SAMPLES:

Contamination must be avoided. Clean the knife for cutting each fish. (Contamination might occur if a cutting tool is used on different fish without cleaning. If contamination occurs, PLEASE annotate this in the sample's information).

B. FREEZING COLLECTION

SAMPLING

Samples collected will be used for genetics (liver, heart and muscle) as well as microconstituent analysis (otoliths). This biological material can be collected together (juveniles) or separately (adults). We request to get the largest possible amount of material.

B.1 JUVENILES.

For juveniles, we recommend to keep a portion of the fish from the head to the middle of the body approximately. This method will preserve the otoliths and the rest of the tissues. Total portion length will depend on fish size (the whole fish is not required since it takes too much space and weight).

B.2. ADULTS.

1. Head containing the otoliths must be removed from the rest of the fish. Please, keep only the head portion which contains the otoliths.
2. From the rest of the fish, cut a portion that includes the three tissues required (**heart, liver and muscle**).
3. All samples must be **frozen**, as soon as possible, after collecting. If this is not possible, you may keep the sample on ice until arriving at the laboratory. Then, keep the samples immediately in the freezer.
4. Tissue extracts must be placed into labeled plastic bags. Please, remember that biological material coming from the same fish must be labeled with the same code. If this fish has been used in other studies (maturity, morphometry,...) please use the same code. Complementary data about age and sampling location are needed.

SHIPPING

1. Samples must be shipped on dry ice. (A stay-foam cooler is sufficient). If you don't have dry ice, alternatively, you can cover up the cooler with ice and bring into the freezer for some days before shipping.
2. Shipment must be carried out by Air Cargo System in regular flight as personal cargo (this way is faster and cheaper than the other deliveries).
3. Send us, by fax, the "Air Waybill" document. This document is necessary for us to pick up the parcel at the Barcelona airport. As personal cargo, I can remove the parcel directly from the Spanish customs, on the same day, 2 hours after the arrival of the flight.
4. Alternatively you must send to ICCAT the invoice of the shipment.

C. ETHANOL COLLECTION

SAMPLING AND SHIPPING

1. From each individual, tissues required are **heart or skeletal muscle**.
2. Cut some pieces of tissue and place it in vial with 96% ethanol (70% ethanol is also enough). **Smaller pieces are better than larger ones, to ensure penetration of ethanol.**
3. Place vial in plastic bag and label.
4. Shipment can be carried out by any mail regular system or other deliveries, to the address below

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