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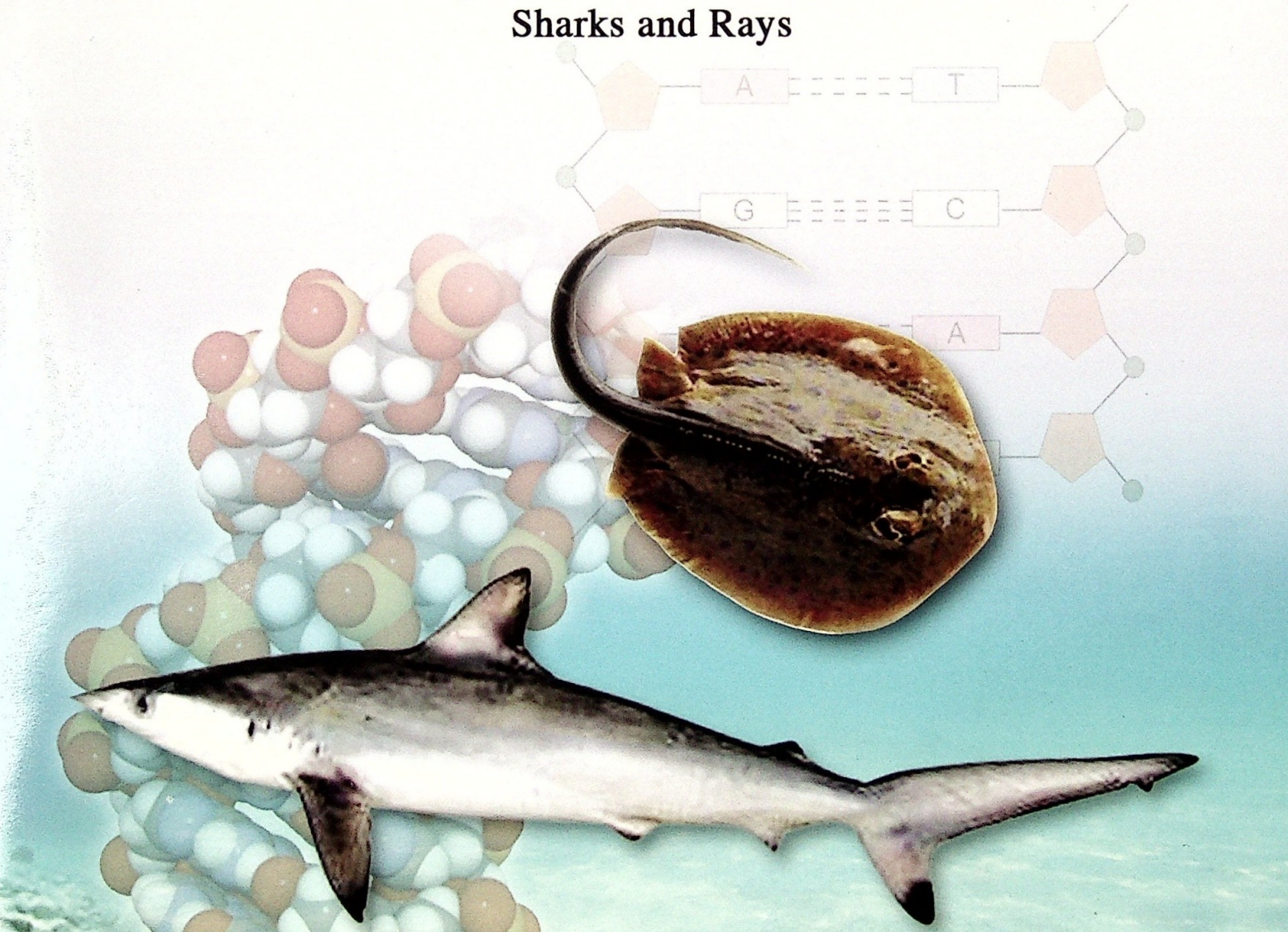
STANDARD OPERATING PROCEDURE

for

Tissue Sample Collection and Preservation of
Sharks and Rays



MARINE FISHERY RESOURCES DEVELOPMENT AND MANAGEMENT DEPARTMENT (MFRDMD)
SOUTHEAST ASIAN FISHERIES DEVELOPMENT CENTER (SEAFDEC)
TAMAN PERIKANAN CHENDERING, 21080 KUALA TERENGGANU, MALAYSIA

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1.0 INTRODUCTION

This Standard Operating Procedures (SOP) serves as a guideline and reference for sharks and rays tissue collection/sampling. Tissue collected is analysed using molecular biology technique as an alternative identification method. Currently DNA barcoding research on sharks is progressing in the world. It is expected that DNA data on some elasmobranch species in the region are available. Compilation of the available data and species identification are performed on sharks and rays caught in Malaysia waters. Genetic research on some of the unsequenced species is also conducted. High biodiversity in the region makes this project sophisticated and more than 170 species of elasmobranches have been recorded. The expected outputs for the project include the biological information of sharks and rays in the region, which can be used for development and management of sharks and rays in the region.

2.0 OBJECTIVE

Study on biology of major elasmobranch (sharks and rays) species, provide basic knowledge for conservation and enhancement of shark and ray populations in the region. More recently, on a regional level the pressure to list commercially important and valuable marine species on CITES is growing. Identification of elasmobranch species is fundamental of biological data collection. Expertise on identification and biological data collection on sharks and rays in the region need to be strengthened.

3.0 SAMPLING AT PORTS

3.1 Point of concern

a. Sampling location. Species identification by authorized taxonomist

b. Fresh sample gives better DNA extraction. Samples collected must be from only properly preserved catch on board of the fishing vessel. This is to ensure freshness of the fish sampled.

c. Need to maintain the freshness of fish until the tissue is sampled and preserved. Sampled fishes at the sampling site should be kept in a container with ice or dry ice to maintain the freshness of the samples until tissue collection and preservation activities is done. This particularly important in the case when tissue collection and preservation activities is to be carried out not only *in situ* (at the sampling site) but also later after taken back to laboratory. (Refer to 4.3). (Note that, once packed of crashed ice/dry ice must be used until tissue preservation is done).

d. Possible cross-contamination when fish are sampled from mix-species container. Ensure sampled fish are properly wiped clean of slim around sample site before tissue sampling.

3.2 Materials and tools for sampling at port

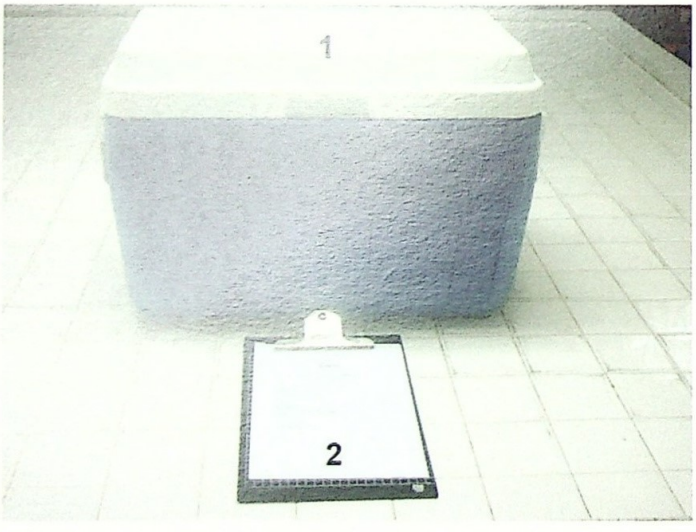


Figure 1: Materials and tools used for sampling at port

Table 1: List of materials and tools for sampling at port

	NAME	DESCRIPTION
1	Cooling box	This is suitable for transportation of sample from sampling port to the laboratory. Its size depending on the sample.
2	Data Form 1	Each sample must be attached a proper identification label (Appendix I).
3	Ice or Dry Ice *	This is one of the important items for genetic sample collection. Sample amount should be prepared for the sample collection.

* Not in the picture

3.3 Procedure for sampling at port

1. Samples collected and species identification at landing site. Include tag for each sample.

   Fill form 1 (Appendix 1)

2. Put the sample into ice box to maintain freshness of the samples.

3. The fish samples should be maintained covered with crash ice or use of dry ice in the ice box until the next step for tissue preservation. Tissue preservation will be done at laboratory.

4. At laboratory, fish samples should be kept in freezer preferably at - 20^0C until tissue preservation procedure is carried out.
*Proceed to 4.3 if the tissue decided to be preserved at the same time.

4.0 TISSUE SAMPLE COLLECTION AND PRESERVATION

4.1 Point of concern

1. Need to maintain the freshness of the sample. Pelvic fin should be taken immediately after the sample fish was taken out from the storage. The remaining ice/water must be wiped out from the sampling area.

2. Avoid contamination of the sample. Forceps and scissors must be washed with clean water and ethanol and burn to sterilize every time before use.

3. Sample storage temperature is no longer an issue. The vials containing tissue sample in buffer (ethanol) can be stored at room temperature. Once preserved in ethanol samples can be stored for many years. Ethanol should be checked periodically for evaporation. Therefore, storage in fridge or freezer will reduce ethanol evaporation.

4. Tissue should be fully preserved. Each tissue sample should be placed in individual vials, approximately 20 mg of tissue for 1.5 ml of ethanol. No more than ¼ tissue to ¾ ethanol by volume, overloading the vials causes the tissue to be poorly preserved.

5. Sample without proper label is problematic. Vials should be labelled with a non-dissolving ethanol resistant marker or printed labels to avoid loss of label.

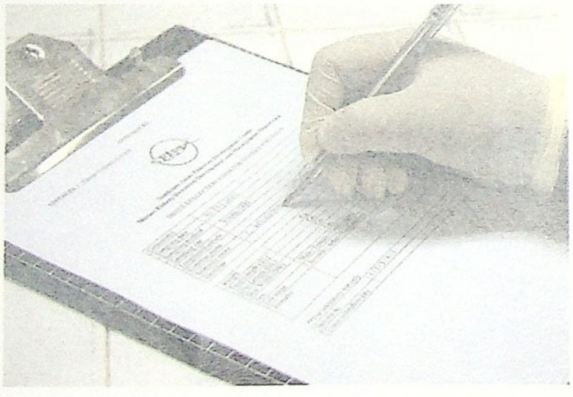
4.2 Materials for tissue collection and preservation

Figure 2: List of materials and tools for tissue collection

Table 2: List of materials and tools for tissue collection

NAME	DESCRIPTION
1. Set of forceps and scissors	Use to cut tissue samples from fish body.
2. Wash bottle filled with ethanol (95%)	Use for wash forceps and scissors.
3. Wash bottle filled with clear water	Use for rinse forceps and scissors.
4. Burner or alcohol lamp or lighter	Use for sterilizing forceps and scissors.
5. Tray	For placing specimen during tissue collection.
6. Vials filled with preservation buffer	In which tissue samples are preserved with buffer contained 95 % ethanol.
7. Tissue paper	To wipe out the water and any organics from forceps and scissors.
8. Disposable gloves	To wear during sampling process.
9. Permanent marker	To label samples.
10. Weight Scale	To weight the specimens.
11. Measuring Tape	To measure the specimens.
12. Form 1 (from port)	To record the information.

4.3 Procedure for tissue collection and preservation



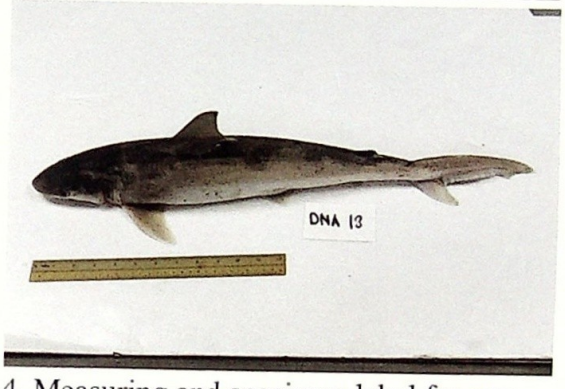
1. Record all information of the specimen into Form 1.

2 Label the vials with format given. (Species/Year/Sample Number). :
The vial should be labelled with species (e.g *Carcharhinus brevipinna* as C. brevipinna), date (year), sampling site and vial number (as the vial number in Form 2).
Example:

Species : C. brevipinna
Year : 2013 = 13
Sampling site: Kuantan
Sample number : 01

Code: **Cb/13/KTN/01**
Proceed to step 3 for sharks and step 4 for ray.

3. Measuring and specimen label for sharks



3a. Take picture of the specimen with scale and tag. Proceed to step 5

4. Measuring and specimen label for rays

4a. Take picture of both surfaces of the specimen with scale and tag. Proceed to step 6.

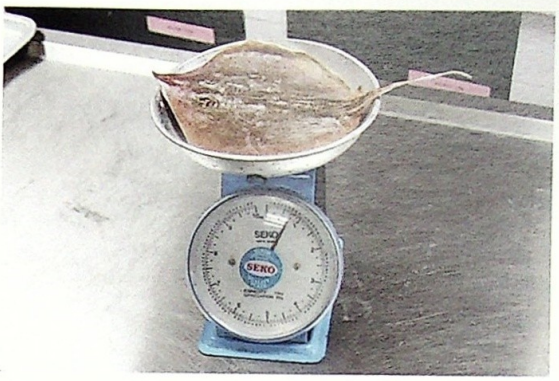
5. Weight for shark

5a. Weigh the specimen and record in Form 1.

5b. Measure total length of the specimen and record in Form 1.

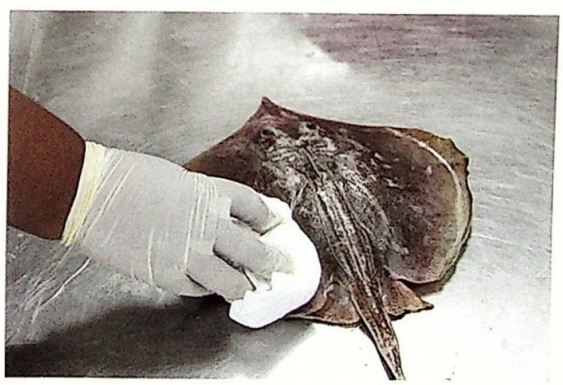
5c. Wipe the sample fish with tissue paper. Proceed to number 7.

6. Weight and measurement for rays



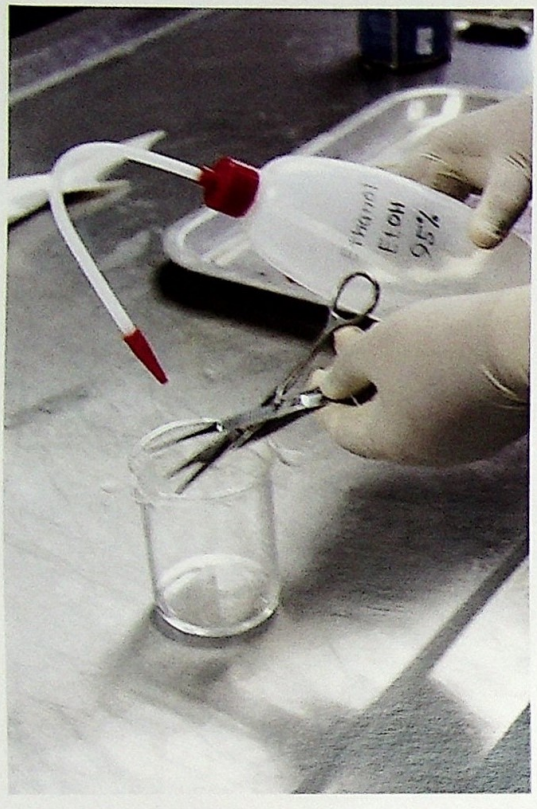
6a. Weigh the specimen and record in Form 1.

6b. Measure total length/disc length/disc width of the specimen and record in Form 1.



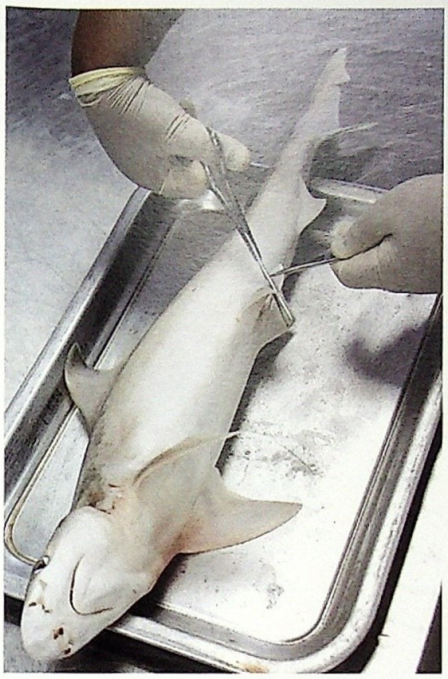
6c. Wipe the sample fish with tissue paper. Proceed to number 7.

7. Wash forcep and scissors with clean water and then wipe with tissue paper.

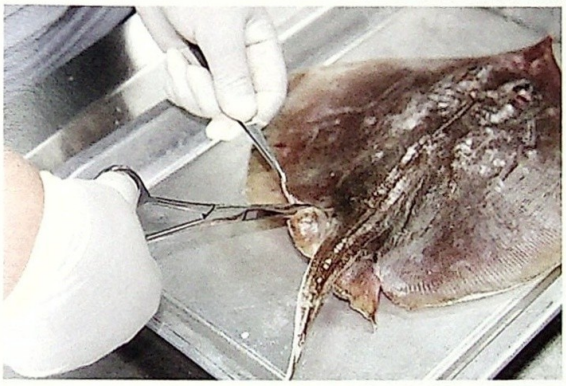


8. Wash forcep and scissors with 95% ethanol.

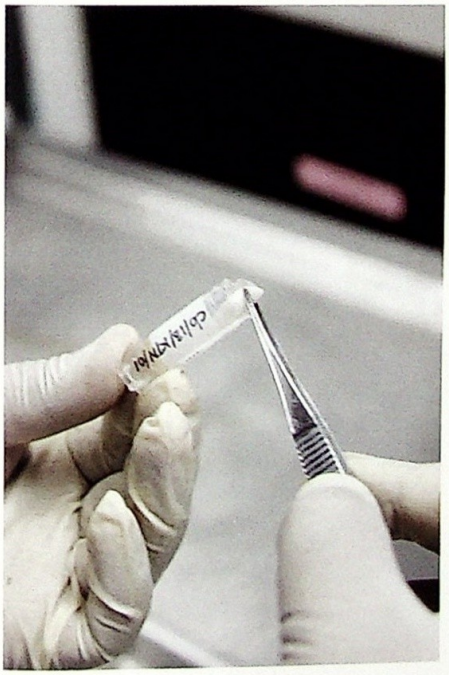
9. Burn scissors and forcep for sterilization. Note: Never touch the edges of sterilized tools.



10. Cut approximately 30 mg of pelvic fin of the fish. Please avoid any contamination. Proceed to number 12 for the shark species.

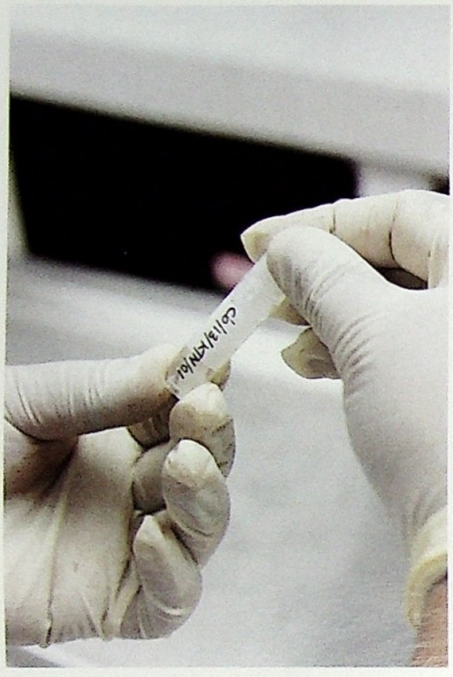


11. Cut approximately 30 mg of pelvic fin of the fish. Please avoid any contamination.



12. Immediately, by using forcep, place the cut tissue into a vial that contained preservation buffer.

    Note: Always handle the tissue using sterilized tools to avoid contamination.



13. Screw the vial cap the vial tightly and place in a safe container.

14. Sterilize the scissors and forceps before taking tissue sample from the next specimen: Repeat steps 1 to 14 for each sample.

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Abu Talib, A., Noorul Azliana, J. & Wahidah, M.A. (2013). Standard Operating Procedure for Tissue Sample Collection and Preservation. SEAFDEC/MFRDMD/SP/21.

Steinke, D. & Hanner, R. (2011). The FISH-BOL Collaborators' Protocol. *Mitochondrial DNA*, 22(S1): 10-14.

APPENDIX I

FORM 1

Tissue Collection Form

Southeast Asian Fisheries Development Center

Marine Fishery Resources Development and Management Department

TISSUE COLLECTION FORM FOR SHARKS AND RAYS

Collector(s)	
Sampling date	
Sampling method	
Sampling site	
Longitude/Latitude	
Common name	
Scientific name	
Identifier	

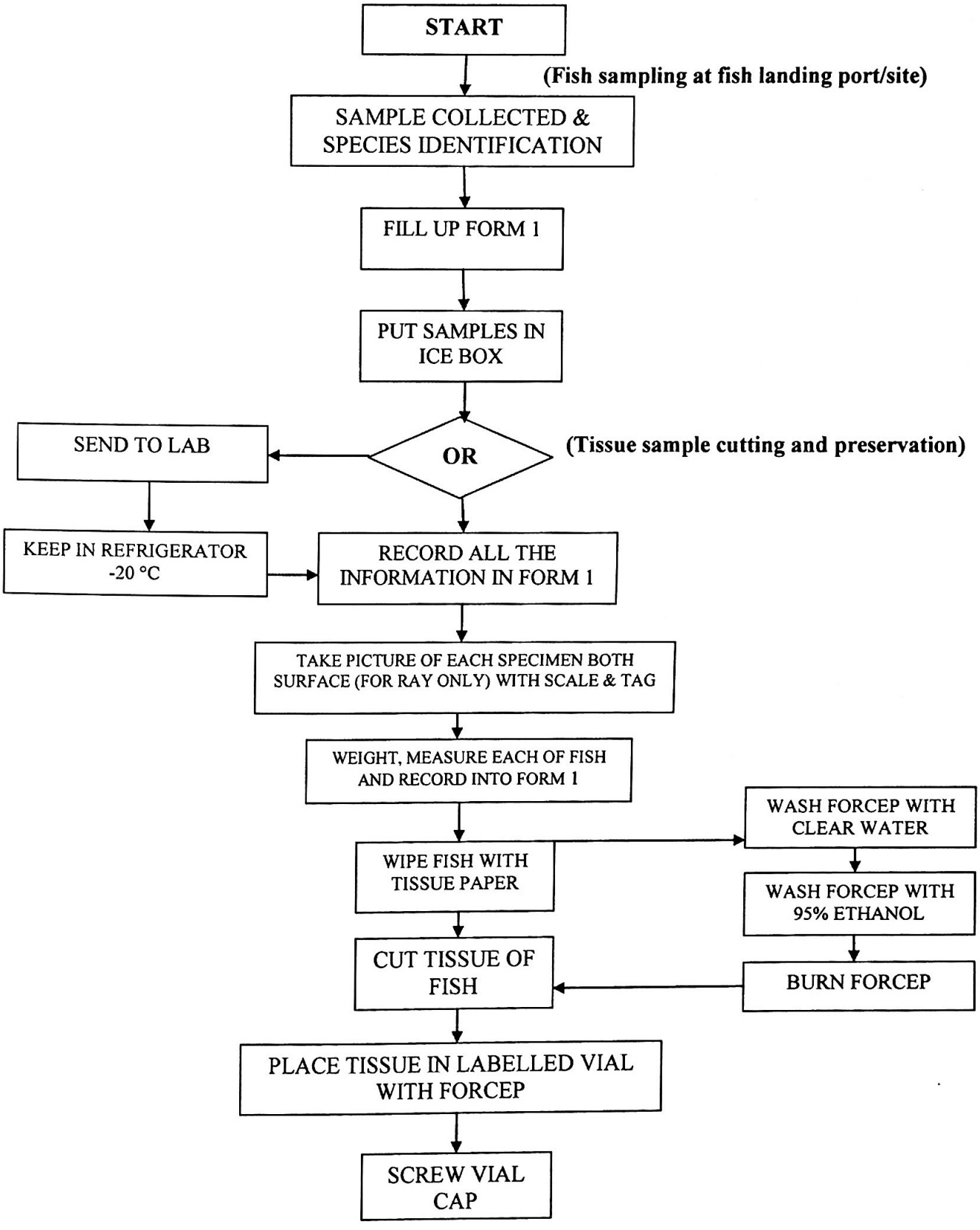
Weight	Kg
Total Length (TL)	Cm
Disc Length (DL)	Cm
Disc Width (DW)	Cm
Sex	Male / Female / Unidentified
Life stage	Juvenile / Adult / Unidentified
Remarks	

**For laboratory use only:

Sample no.	1 / 2 / 3 / 4 / 5
Tissue collection date	

APPENDIX II

Flow Chart for Tissue Sample Collection Procedure



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