Genetic Survey for Population Structure
Program for Economically Important Pelagic Species in the South China Sea and Andaman Sea:

Standard Operating Procedure for Tissue Sample Collection and Preservation

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Introduction

This SOP will be used as a guideline and reference by researchers and enumerators in the field for tissue sample collection and preservation for stock/population identification.

Objectives

To standardize the tissue sample collection of the specimen of *Rastrelliger kanagurta* and *Decapterus maruadsi* in the Southeast Asian region for genetic study. This is necessary to obtain reliable data and comparable data for stock/population clarification covering the whole of South China Sea and Andaman Sea areas.

Target Species:

Indian mackerel (*Rastrelliger kanagurta*) and Japanese scad (*Decapterus maruadsi*), the two most common of small pelagic fish species in the region.

Identification of Species

It is recommended that the identification of fish species is based on the reference book entitles, *Field Guide to Important Commercial Marine Fishes of the South China Sea published by SEAFDEC-MFRDMD/SP/2.*

a) *Rastrelliger kanagurta* (Indian mackerel)

b) *Decapterus maruadsi* (Japanese scad)

Figure 1: Targeted species in this study

[Type text

Sampling areas

It was agreed that the sampling sites selected were as listed in Table 1.

Table 1: Sampling sites and number of samples to be collected covering both the South China Sea and the Andaman Sea.

No.	Sampling site	No. of samples	No. of species	Total
1.	Muara, Brunei Darussalam	35	2	70
2.	Sihanouk Ville, Cambodia	35	2	70
3.	Yangon, Myanmar	35	1*	35
4.	Kuantan, Malaysia	35	2	70
5.	Kuching, Malaysia	35	2	70
6.	Kudat, Malaysia	35	2	70
7.	Pangkor, Malaysia	35	1*	35
8.	Banda Acheh, Indonesia	35	1*	35
9.	Pekalongan, Indonesia	35	2	70
10.	Rosario, Phillipines	35	2	70
11.	Ranong, Thailand	35	1*	35
12.	Songkhla, Thailand	35	2	70
13.	Khanh Hoa, Vietnam	35	2	70
14.	Nghe An, Vietnam	35	2	70

Remark:
* Only *Rastrelliger kanagurta*

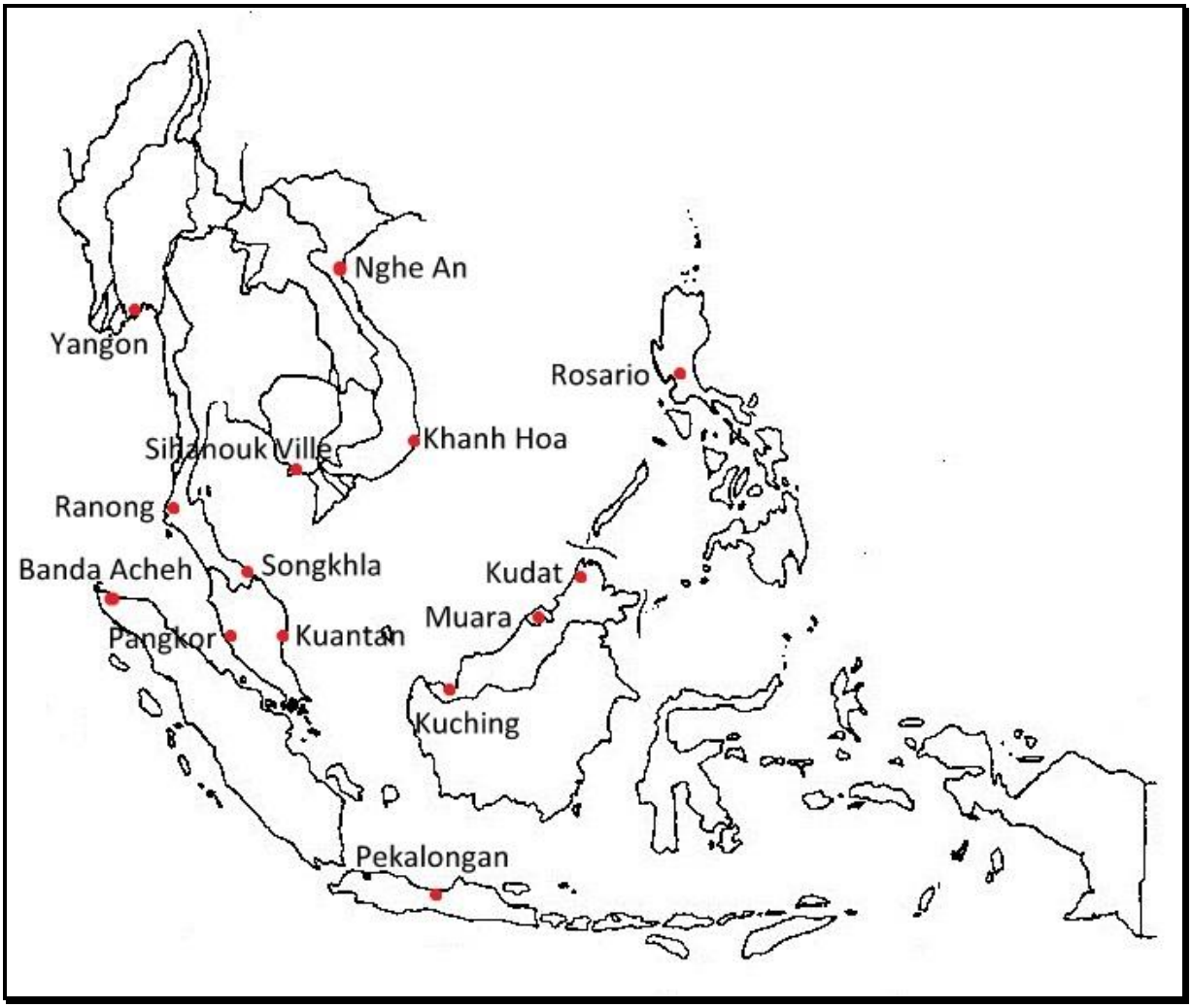


Figure 2: Map showing the distribution of the sampling sites in the South China Sea and the Andaman Sea.

1.0 SAMPLING AT PORTS

1.1 Consideration points

1. Fish samples **must be collected at the landing site**. This is to ensure to get the fresh samples. The samples must be taken from the catch of as many size categories of vessels (small, medium and large) or fishing zones as possible.

2. For each species, at least 35 individuals are required. For example, if there are three types of gear to be sampled, a total of 12 individuals must be collected from each gear type. This 12 fishes as much as possible must be collected from various vessel categories (small, medium and large or fishing zones) of that particular gear type.

3. **The freshness of fish is very important for genetic study**.
   To keep the sample fresh, ice or dry ice must be used until tissue preservation is done. Tissue preservation can be carried either at landing site or at laboratory. (Refer to 2.2)

1.2 Materials and tools preparation for sampling at port

Figure 3: Materials and tools used for sampling at port

Table 2: List of materials and tools for sampling at port

	NAME	DESCRIPTION
1	Plastic bag *	This is used for sample packaging, the size is depending on the fish size to be collected
2	Cooling box *	This is suitable for transportation of sample from sampling port to the laboratory. Its size depending on the sample.
3	Disposable gloves	To wear during sampling process.
4	Data Form 1	Each sample must be attached a proper identification label (Appendix I).
5	Ice or Dry Ice *	This is one of the important items for genetic sample collection. Ample amount should be prepared for the sample collection.

Remarks:
1. * Are not supply by MFRDMD.
2. All materials and tools as shown in the Figure 2 except item number 5.

1.3 Procedure for sampling at the port

1. Samples collected at the identified landing sites from different vessel category and gear type (to ensure the whole fishing area coverage). The sample should be packed separately by gear type and vessel category and accompanied with filled up Form 1.

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

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 could be done either at the landing site or after the samples are brought back to laboratory.

   *Please proceed to 2.3 if tissue is decided to be preserved in-situ (at the same landing site).

4. At laboratory, fish samples should be kept in freezer preferably at -20^{o}C until tissue preservation procedures is carried out.

2.0 TISSUE SAMPLE COLLECTION AND PRESERVATION PROCEDURE

2.1 Consideration points

1. Muscle tissue should be taken immediately after the sample fish was taken out from the storage.

2. It is recommended that the tissue is taken from the dorsal part of the fish body.

3. Forceps must be washed with clean water and ethanol and burn to sterilize every time before use.

4. The vials should be labeled with same number as recorded with Form 2 (Tissue Samples Collection Form).

5. The vials containing tissue sample in buffer can be stored at room temperature.

2.2 Materials and tools preparation for tissue sample collection

Figure 4: List of materials and tools for tissue collection

Table 3: List of materials and tools for tissue collection

NAME	DESCRIPTION
1. Set of forceps and scalpels	Use to cut tissue samples from fish body.
2.Wash bottle filled with ethanol (95%)*	Use for wash forceps and scalpels.
3. Wash bottle filled with clear water	Use for rinse forceps and scalpels.

4. Burner or alcohol lamp or lighter*	Use for sterilizing forceps and scalpels.
5. Tray*	For placing specimen during tissue collection.
6. Vials filled with preservation buffer	In which tissue samples are preserved with buffer contained 20 % DMSO.
7. Tissue paper*	To wipe out the water and any organics from forceps and scalpels.
8. Disposable gloves	To wear during sampling process.
9. Permanent marker	To label samples.
10. Data Form 2	Information for one species must be fill up in the same form.

Remark:
* Items are not supply by MFRDMD

2.3 Procedure for tissue cutting and preservation

1. Transfer information about the sample from Form 1 into Form 2.

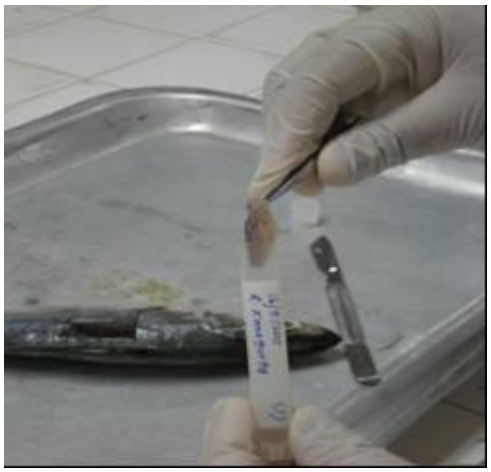
2. Wipe the sample fish with tissue paper.

3. Wash forceps and scalpels with clean water and then wipe with tissue paper.

4. Wash forceps and scalpels with 95% ethanol.

5. Burn forceps and scalpels for sterilization.
   Note: Never touch the edges of sterilized tools.

6. Cut approximately 1 cm^3 (1cm x1cm x 1cm) of muscle tissue from the dorsal part of the fish. During the cutting, please ensure the abdomen part of the fish is not cut. This is to avoid contamination of blood and stomach contents.



7. Immediately, by using forceps, place the cut tissue into a vial that contained preservation buffer. The vial should be labeled with sampling area (e.g. Kuantan), species (e.g. RK for *Rastrelliger kanagurta*), date (dd/mm/yy) and vial number (as the vial number in Form 2).

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

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

9. Change the blade of the scalpel before taking tissue sample from the next specimen: Repeat steps 1 to 9.

3.0 TRANSPORTATION OF THE VIALS TO MFRDMD

Bunch all the vials together using rubber band and wrap the bundle with air bubble plastic provided before placing it in a mail box provided.

Technical officer is required to send all the samples to MFRDMD using courier service (e.g. DHL, FEDEX, etc.).

MFRDMD will notify member country upon receiving of the parcel.

REFERENCES

Mat Isa, M. & et.al. 2004. Standard Operating Procedures for data collection and analysis, Information Collection for Sustainable Pelagic Fisheries in the South China Sea, SEAFDEC, Kuala Terengganu ,

Ref: Mansor, M.I., Kohno, H., Ida, H., Nakamura, H.T., Aznan, Z. and Abdullah, S. 1998. Field Guide to Imp[ortant commercial Marine Fishes of the South China Sea. SEAFDEC MFRDMD/SP/2.

APPENDIX I : Form 1

Southeast Asian Fisheries Development Center
Marine Fisheries Resources Development and Management Department

Form 1: Fish Samples Collection Form

Country:

Sampling area:

Date :

Species :

Type of gear :

Vessel category/ Fishing zone :

No. of Samples :

APPENDIX II : Form 2

Southeast Asian Fisheries Development Center
Marine Fisheries Resources Development and Management Department

Form 2 : Tissue Samples Collection Form

Country :	**Sampling area :**
Species :	**Total number of samples :**
Technical Officer In Charge :	
Agency :	
E-mail Address :	**Contact No. :**

Vial No.	Date of Sampling	Type of gear	Vessel category/fishing zone	Remark/s
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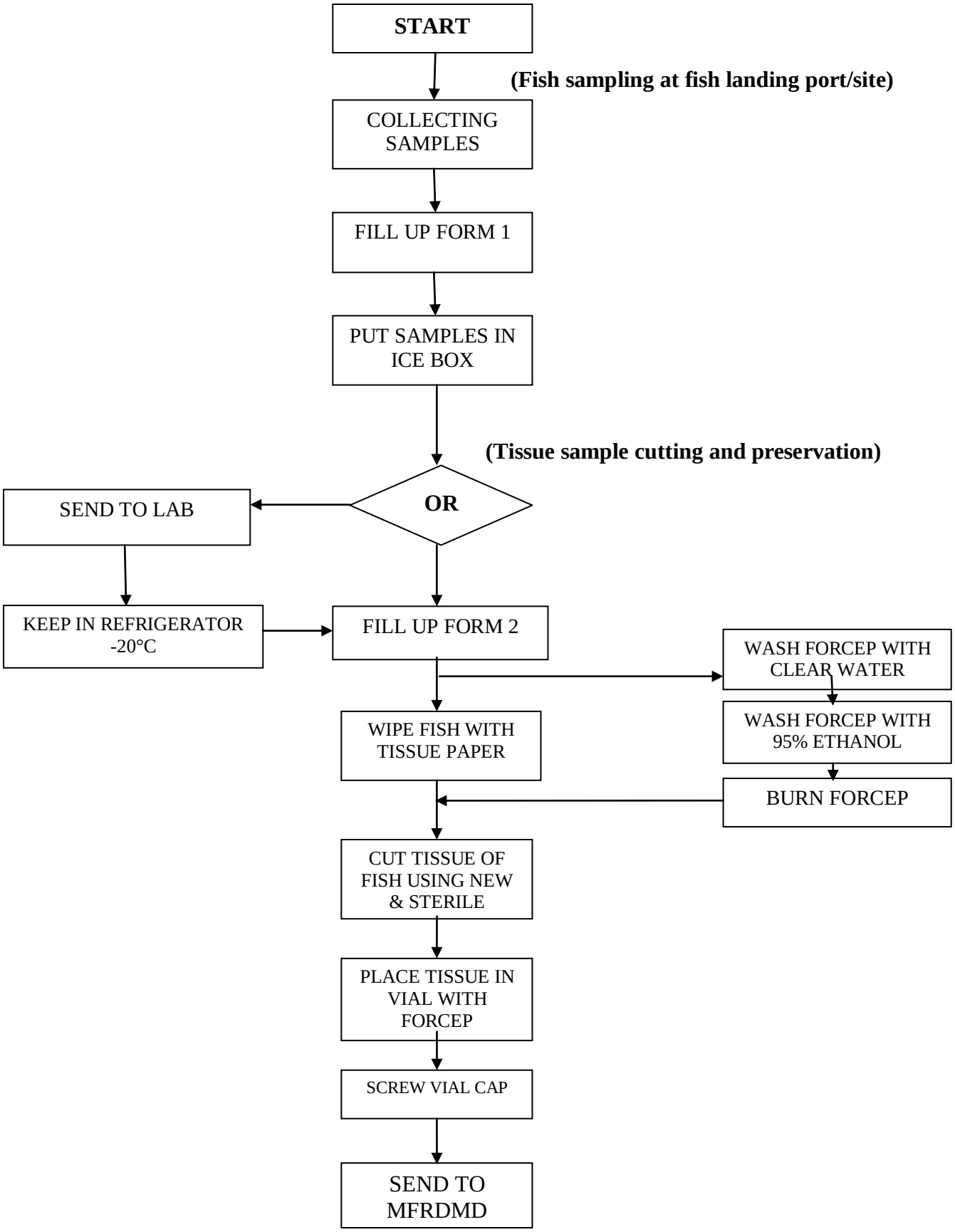
APPENDIX III : FLOW CHART

Flow Chart for Tissue Sample Collection Procedure



APPENDIX IV: TAXONOMY KEYS

Key to Species of Mackerels (pg. 99)

Key to species of genus *Rastrelliger*

1 Body deep; no dusky stripes along sides of body (Fig. 641) ..... ***Rastrelliger brachysoma***
Body not deep; dusky stripes running along sides of body (Fig. 642) **2**

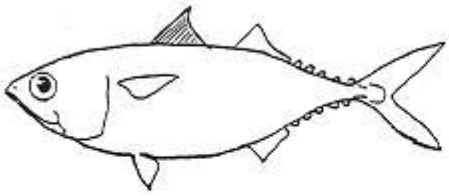


Figure 641.

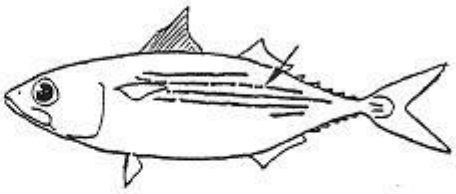


Figure 642.

2(1) Gill rakers small in number, 20-25 (Fig. 643) ..... ***Rastrelliger faughni***
Gill rakers more than 30 ..... ***Rastrelliger kanagurta***

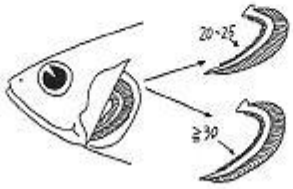


Figure 643.

Key to Species of Scads (pg. 55-56)

Key to species of genus *Decapterus*

1 Scutes present on the whole straight portion of lateral line (Fig. 298) **2**
Scutes present on less than 2/3 of the straight portion of lateral line (Fig. 299) **4**

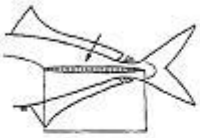


Figure 298.

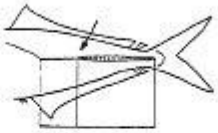


Figure 299.

2(1) Pectoral fin very long, reached below origin of soft dorsal fin; tail red; body depth more than 1/4 FL (Fig. 300) ..... ***Decapterus akaadsi***
Pectoral fin long, extending to end of first dorsal fin; tail not red (Fig. 301) **3**

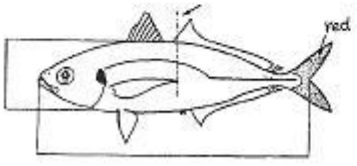


Figure 300.

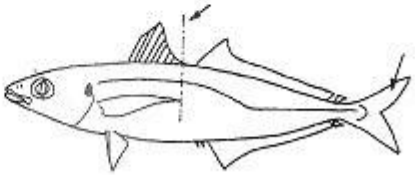


Figure 301.

3(2) Body slender, its depth about 1/4 FL; predorsal scales not extending to interorbital space (Fig. 302) ..... ***Decapterus russelli***

Body rather high, body depth about 1/4 FL; predorsal scales extending to above anterior rim of eyes (Fig. 303) ..... ***Decapterus maruadsi***

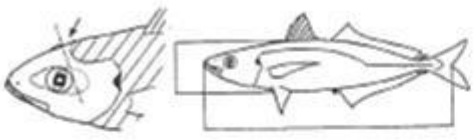


Figure 302.

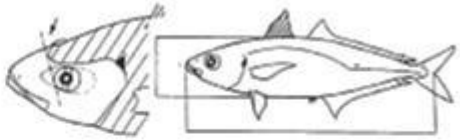


Figure 303.

4 Pectoral fins very short, ending below mid-first dorsal fin; predorsal scales not extending to interorbital space (Fig. 304) ..... ***Decapterus macrosoma***

Pectoral fins short, reaching below end of first dorsal fin; predorsal scales reached interorbital space (Fig. 305) **5**

Figure 304.

Figure 305.

5 Mouth floor uniformly dark (Fig. 306) ..... ***Decapterus muroadsi***

Posterior half of mouth floor whitish (Fig. 307) ..... ***Decapterus macarellus***

Figure 306.

Figure 307.