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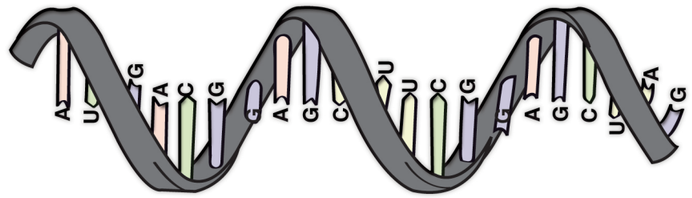
STANDARD OPERATING PROCEDURES (SOP)

FOR
TISSUE SAMPLE COLLECTION AND PRESERVATION



"GENETIC SURVEY FOR POPULATION STRUCTURE PROGRAM FOR ECONOMICALLY IMPORTANT PELAGIC SPECIES IN THE SOUTH CHINA SEA AND ANDAMAN SEA"

CONDUCTED BY
SOUTHEAST ASIAN FISHERIES DEVELOPMENT CENTER/
MARINE FISHERY RESOURCES DEVELOPMENT AND MANAGEMENT DEPARTMENT

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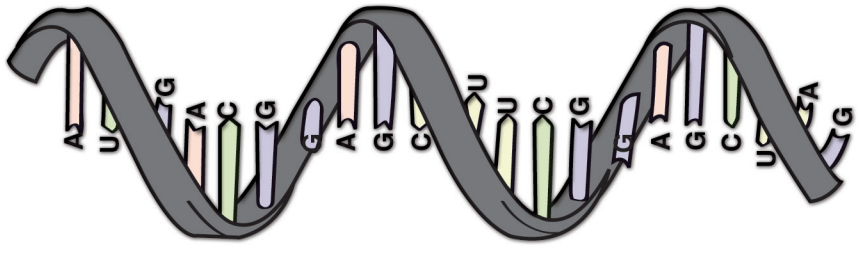

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TABLE OF CONTENTS

List of Tables

List of Figures

List of Appendices

1. INTRODUCTION

This Standard Operating Procedure (SOP) serves as a guideline and main reference for those involve in tissue sample collection in the field at identified sampling/landing sites (Table 1) and tissue preservation either in the field or at laboratory. Collected and preserved samples from the respective country are to be sent via air courier to SEAFDEC/MFRDMD in Malaysia for further laboratory works.

2. OBJECTIVE OF THE SOP

The main objectives of this SOP are to standardized sampling procedure and ensure all collected tissues are prepared and preserved following the same methods and procedures. The steps outlined in the SOP ensure sufficient high quality DNA could be obtained from the sampled tissues. The high quality DNA is required to produce reliable and comparable data for stock/population clarification covering the whole large ecosystem of South China Sea and Andaman Sea areas.

3. TARGET SPECIES

1. *Rastrelliger kanagurta* (Cuvier, 1816), Indian mackerel.

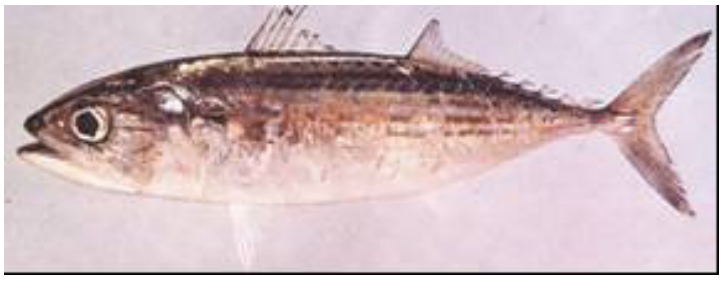


Figure 1: Indian mackerel (*Rastrelliger kanagurta*) is the target species for genetic stock study in Andaman Sea and South China Sea ecosystem

2. *Decapterus maruadsi* (Temminck & Schlegel, 1843), Japanese scad.

Figure 2: Japanese scad (*Decapterus maruadsi*) is the target species for genetic stock study in South China Sea ecosystem

Identification of Species

It is very important to be sure that fish sampled is *Rastrelliger kanagurta* (Figure 1) and *Decapterus maruadsi* (Figure 2).

The key to genus *Rastrelliger* and *Decapterus* of any taxonomic book would serve a good reference. The scanned page on 'The key to genus *Rastrelliger'* from published book by SEAFDEC/ MFRDMD entitled; *Field Guide to Important Commercial Marine Fishes of the South China Sea,* is given below for quick reference (Figures 3, 4 and 5).

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**

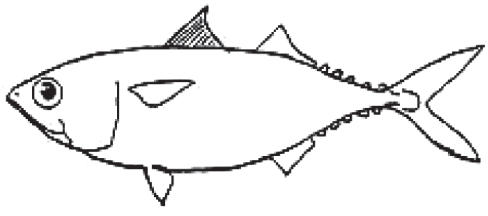


Figure 641.

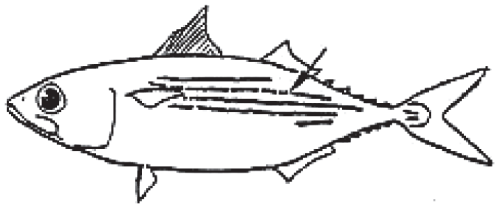


Figure 642.

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

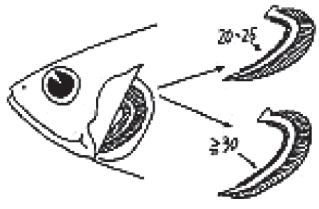


Figure 643.

Figure 3 : Taxonomic key to distinguish Indian mackerel from other species under genus *Rastrelliger*

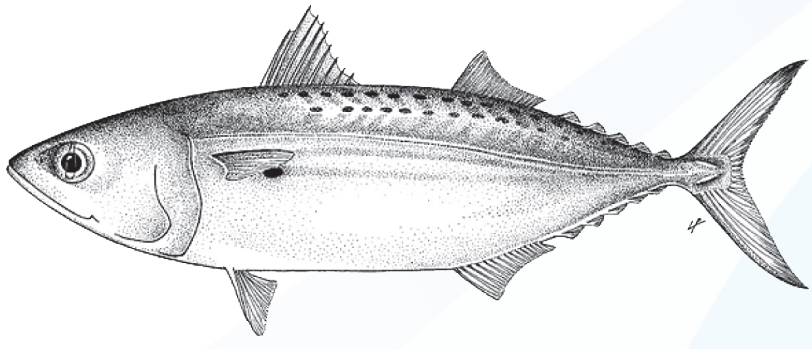


Figure 4: *Rastrelliger kanagurta* showing the dusky stripes running along both sides of the body. (cited from FAO Species Identification Sheets)

.

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

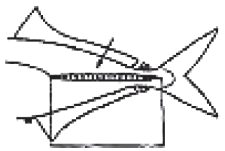


Figure 298.

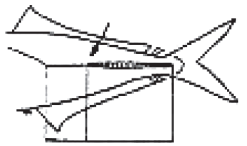


Figure 299.

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**

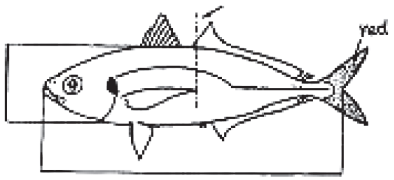


Figure 300.

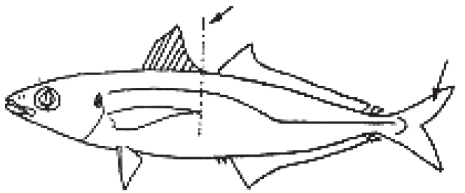


Figure 301.

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***

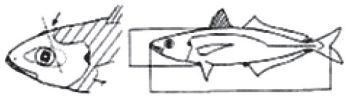


Figure 302.

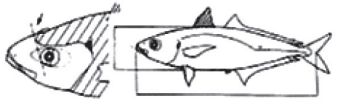


Figure 303

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.

Figure 5: Taxonomic key to distinguish Japanese scad from other species under genus *Decapterus*

4. SAMPLING AREAS

The complete list of sampling sites as shown in Table 1 and Figure 6.

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	Species	Total
1.	Muara, Brunei Darussalam	35	*Rastrelliger kanagurta* *Decapterus maruadsi*	70
2.	Sihanouk Ville, Cambodia	35	*Rastrelliger kanagurta* *Decapterus maruadsi*	70
3.	Yangon, Myanmar	35	*Rastrelliger kanagurta*	35
4.	Kuantan, Malaysia	35	*Rastrelliger kanagurta* *Decapterus maruadsi*	70
5.	Kuching, Malaysia	35	*Rastrelliger kanagurta* *Decapterus maruadsi*	70
6.	Kudat, Malaysia	35	*Rastrelliger kanagurta* *Decapterus maruadsi*	70
7.	Pangkor, Malaysia	35	*Rastrelliger kanagurta*	35
8.	Banda Acheh, Indonesia	35	*Rastrelliger kanagurta*	35
9.	Pekalongan, Indonesia	35	*Rastrelliger kanagurta* *Decapterus maruadsi*	70
10.	Bataan, Phillipines	35	*Rastrelliger kanagurta* *Decapterus maruadsi*	70
11.	Palawan, Phillipines	35	*Rastrelliger kanagurta* *Decapterus maruadsi*	70
12.	Ranong, Thailand	35	*Rastrelliger kanagurta*	35
13.	Songkhla, Thailand	35	*Rastrelliger kanagurta* *Decapterus maruadsi*	70
14.	Khanh Hoa, Vietnam	35	*Rastrelliger kanagurta* *Decapterus maruadsi*	70
15.	Nghe An, Vietnam	35	*Rastrelliger kanagurta* *Decapterus maruadsi*	70

Figure 6: Map showing the distribution of the sampling sites in the South China Sea and the Andaman Sea.

5. SAMPLING AT PORTS

5.1 Points of concern

a. Fish samples **must be collected at the landing site listed in the Table 1**. Information on where the fish was caught is crucial to ensure the samples represent the fishing area is intended.

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. Avoid other than the target species in the 35 sampled tissues. To ensure only target species is sampled, sampled fish should be larger than 16 cm in size (standard length) for Indian mackerel (*R. kanagurta*) are common caught with Short mackerel (*R. brachysoma*). The Taxonomic key is not works well for smaller size fish.

d. 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 in situ (at the sampling site) but later after taken back to laboratory. (Refer to 6.2). (Note that, once packed of crashed ice/dry ice must be used until tissue preservation is done).

e. 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.

5.2 Materials and tools preparation for sampling at port

Figure 7: 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.

5.3 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 6.0 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.

6.0 TISSUE SAMPLE COLLECTION AND PRESERVATION PROCEDURE

6.1 Points of concern

1. Need to maintain the freshness of the sample. Muscle tissue 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 (the dorsal part of fish).

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. Avoid mixing of samples. The vials should be labeled clearly according to the format given (Species/Year/SampleNumber). E.g. Brunei Darussalam (RK/2011/01). The vials should be labeled with same number as recorded with Form 2 (Tissue Samples Collection Form).

4. 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.

5. Tissue should be fully preserved. Each tissue sample should be placed in individual vials, approximately 20mg 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.

6. 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.

6.2 Materials and tools preparation for tissue sample collection

Figure 8: 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

6.3 Procedure for tissue cutting and preservation

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

2. Label the vials with format given. (Species/ Year//SampleNumber). :
The vial should be labeled with sampling area (e.g. Kuantan), species (e.g. RK for *Rastrelliger kanagurta*) or DM for *Decapterus maruadsi*), date (dd/mm/yy) and vial number (as the vial number in Form 2).
Example:

Species : R. kanagurta (RK)
Year: 2011 = 11
Sample number : 001 (Ref. Form 2)
Code**: RK/11/001**

Species : D. maruadsi (DM)
Year: 2011 = 11
Sample number : 001 (Ref. Form 2)
Code**: DM/11/001**

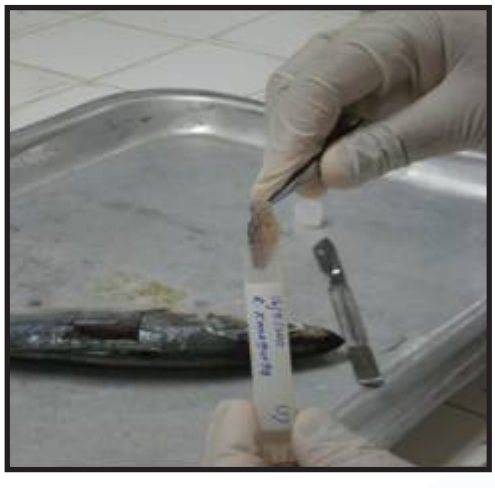
3. Wipe the sample fish with tissue paper.

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

5. Wash forceps and scalpels with 95% ethanol.

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

7. Cut approximately 0.5 cm^3 (1cm x1cm x 0.5 cm) 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.



8. Immediately, by using forceps, place the cut tissue into a vial that contained preservation buffer.
Note: Always handle the tissue using sterilized tools to avoid contamination.

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

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

7.0 TRANSPORTATION OF THE VIALS TO MFRDMD

When: Once 35 samples was obtained.

How to prepare the samples:

i). Shipping samples requires draining ethanol before shipping however **please ensure that the tissue is still remain in a wet form**, (or alternatively replaced ethanol with non-combustible DMSO solution – if available).

ii). Bunch all the vials together using rubber band and wrap the bundle with air bubble plastic provided and seal the plastic bag.

iii). Placing the plastic bag in a postage box (provided).

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

SEAFDEC/MFRDMD will notify member country upon receiving of the parcel.

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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 :

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
1.				
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3.				
4.				
5.				
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Flow Chart for Tissue Sample Collection Procedure