National Fisheries Research and Development Institute
Genetic Fingerprinting Laboratory

UNDP/GEF Sulu-Celebes Seas Sustainable Fisheries Management Project: GENETIC POPULATION STRUCTURE OF SOME PELAGIC FISHES IN THE SULU-CELEBES SEAS

Species Identification and Tissue Sampling Manual

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PREFACE

This manual was made under the UNDP/GEF Sulu-Celebes Seas Sustainable Fisheries Management Project: Genetic Population Structure of Some Pelagic Fishers in the Sulu-celebes Seas. It serves as a basic guide to species identification and tissue sampling which are prerequisites for other biological techniques such as DNA extraction. These two steps greatly affect the end result of an experiment and should therefore be performed properly and accurately. The manual aims to assist researchers and technicians performing the techniques in a simple approach.

The first part of the manual focuses on species identification of four commercially important small pelagics: *Auxis thazard, Rastrelliger kanagurta, Sardinella lemuru* and *Selar crumenophthalmus.* Key features of the said species were summarized, and their differences from morphologically similar species were elaborated.

The second part of the manual provides a step by step guide in performing tissue sampling which includes the appropriate cleaning reagents, materials to be used, proper documentation and the procedure itself.

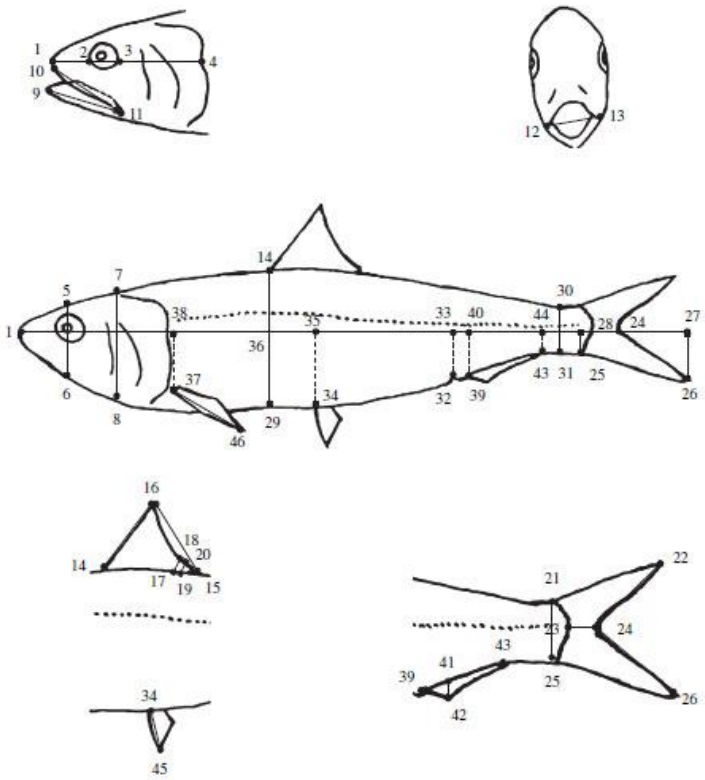
The manual is made possible by the support and efforts from the National Fisheries Research and Development Institute (NFRDI), Genetic Fingerprinting Laboratory, and lastly the Global Environment Facility and the United Nations Development Program.

CONTENTS

SPECIES IDENTIFICATION

Species identification is an important tool in every study in biology. Identification based on morphology is a basic yet hard task especially for closely related species since they look very alike. Misidentification may lead to unwanted outcomes and affects the credibility of one's result, therefore such task should be performed meticulously.

In species identification, it is essential to carefully look at the distinctive characteristics of a species that separates them from a closely related species. Shown below are some key features to look at when identifying a certain species of fish.



1–2	pre orbital length (preOL)
2–3	horizontal diameter of the eye (hDe)
3–4	post orbital length (posOL)
1–4	head length (hL)
5–6	orbital depth of the head (orDH)
7–8	occipital depth of the head (ocDH)
9–11	lower jaw length (ljL)
10–11	maxilliary length (mL)
12–13	maxilliary width (mW)
14–15	dorsal fin base length (dfL)
14–16	length of the first ray of the dorsal fin (L1adf)
17–18	length of the fourth ray from the posterior end of the dorsal fin (L4pdf)
19–20	length of the third ray from the posterior end of the dorsal fin (L3pdf)
21–22	length of the upper lobe of the caudal fin (Lulcf)
23–24	length of the middle lobe of the caudal fin (Lmlcf)
25–26	length of the lower lobe of the caudal fin (Lllcf)
1–27	maximum total length (tL)
1–24	fork length (fL)
1–28	maximum standard length (sL)
14–29	maximum body depth (maxbd)
30–31	minimum body depth (minbd)
1–33	preanal distance (paD)
1–35	preventral distance (pvD)
1–36	predorsal distance (pdD)
35–37	pectoral-ventral distance (p-vD)
35–40	ventral-anal distance (v-aD)
28–40	anal-caudal distance (a-cD)
28–44	length of caudal peduncle (Lcp)
41–42	height of anal fin (Haf)
39–43	length of anal fin base (Lafb)
34–45	length of ventral fin (Lvf)
37–46	length of pectoral fin (Lpf)

Fig. 1. Morphological features commonly used in species identification[1]

Auxis thazard

The genus *Auxis* is characterized as having fusiform, elongated and rounded body. Scales are only present on the corselet but not in the rest of the body. They have two dorsal fins separated by a large interspace with the first being large having ten spines while the second is small with 6 to 9 finlets on its posterior[2].

Auxis thazard, commonly known as frigate tuna, is bluish in color above with oblique black bars above and behind the corselet. A distinction between *Auxis thazard* and a morphologically similar species *Auxis rochei* can be made by observing the corselet, the former having a narrow posterior while the latter having it wide at the posterior[5].This can be seen in figures 2a and 2b.

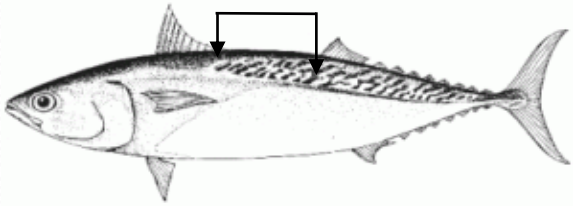


Fig. 2a. Diagrammatic representation of *Auxis thazard* with arrows pointing at the corselet's narrow posterior

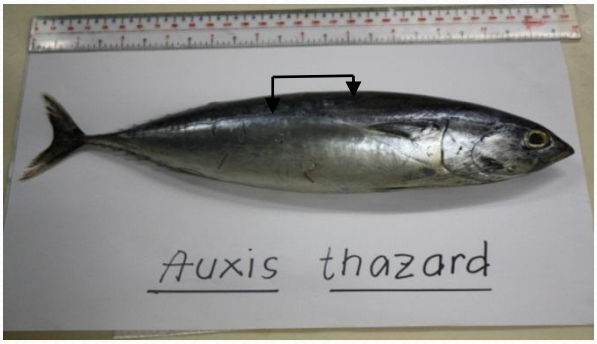


Fig. 2b. Actual picture of *Auxis thazard* with arrows pointing at the corselet's narrow posterior

Auxis thazard

Class — Actinopterygii

Order — others / Perciformes

Finlets absent — Finlets present

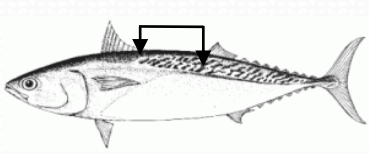
Family — others / Scombridae

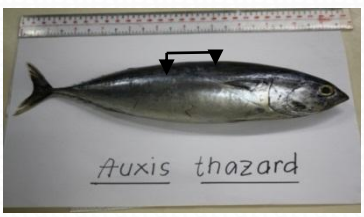
First and second dorsal fins widely Separated, equal to base of first dorsal

Genus — *Auxis* / others

Coslet with narrow posterior — Corslet with thicker posterior

Auxis thazard / *Auxis rochei*





Rastrelliger kanagurta

Rastrelliger is a genus of family Scombridae characterized by moderately deep body. It has eyes covered by an adipose eyelid. It has 2 widely separated dorsal fins with the first having 8-11 spines, and the second being small followed by 5 to 6 anal finlets[6].

Rastrelliger kanagurta is one of the 3 genus of *Rastrelliger* which is commonly known as indian mackerel. It is easily distinguishable from the two other species *R.faughni* and *R.brachysoma* by observing the black spot near the lower margin of the pectoral fins as seen in figures 3a and 3b, and its long gill rakers which can be readily seen by opening its mouth[5].

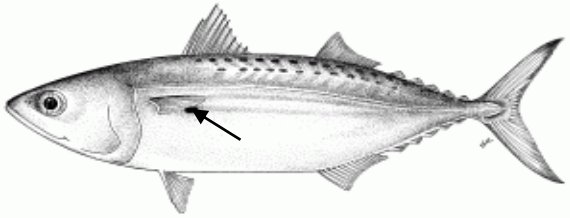


Fig 3a. Diagrammatic representation of *Rastrelliger kanagurta* with the arrow pointing at the black spot near the pectoral fins

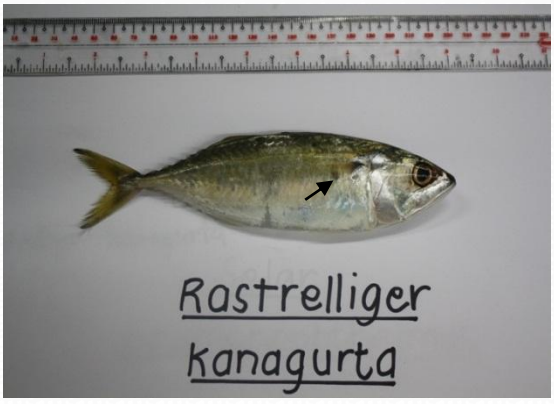


Fig 3a. Actual picture of *Rastrelliger kanagurta* with the arrow pointing at the black spot near the pectoral fins

Rastrelliger kanagurta

Class: Actinopterygii

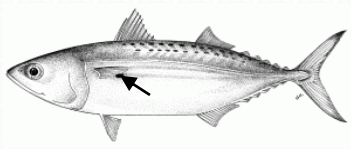
Order: others / Perciformes

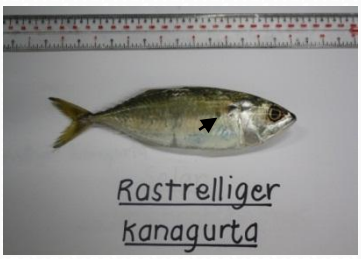
Family: Finlets Absent: others / Finlets present: Scombridae

Genus: 2 horizontal rows of spots on each side of back: *Rastrelliger* / others

Black spot near pectoral fins present: ***Rastrelliger kanagurta*** / *Rasterlliger faughni*

Black spot near pectoral fins absent: *Rastrelliger brachysoma*





Sardinella lemuru

Fishes of genus *Sardinella* have bodies moderately to strongly compressed with the latter forms the belly with very sharp keelf of scutes. Many species under this genera are strongly migratory[7].

One characteristic of *Sardinella lemuru* which distinguishes it easily from other species under the genus *Sardinella* can be seen by looking at the number of fin rays of its pectoral fins. Each pectoral fin has 9 fin rays unlike other species of *Sardinella* having only 8. Figure 4b shows the 9 fin rays of *S.lemuru*[5].

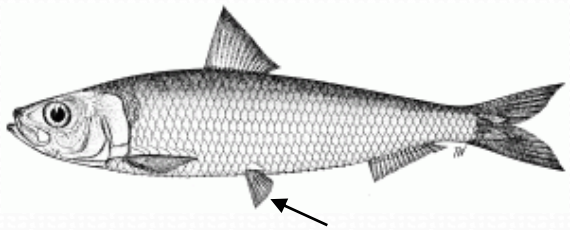


Fig. 4a. Diagrammatic representation of *Sardinella lemuru* with an arrow pointing at the pectoral fins

Fig. 4b. Actual picture of *Sardinella lemuru* with an arrow pointing at the pectoral fins and a closer view of the fin where the 9 fin rays can be observed

Sardinella lemuru

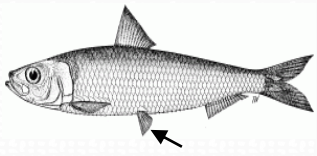
Class — Actinopterygii

Order — others / Clupeiformes

Family — Prepelvic scutes absent: others / Prepelvic scutes present: Clupeidae

Genus — Frontoparietal striae many (7-14): *Sardinella* / others

9 fin rays: ***Sardinella lemuru*** / 8 fin rays: Other *Sardinenlla* species



Selar crumenophthalmus

Species falling under the genus *Selar* have elongated and moderately compressed body. They can also be distinguished by the presence of scutes in a lateral straight line. Their dorsal and anal fins are close to one another[8].

Selar crumenophthalmus is commonly known as big eye scad. As its name implies, it has very large and prominent eyes. The species has relatively small scutes extending shortly from the caudal fin which distinguishes it from *Selar boops*, a similar species in terms of morphology, having relatively big scutes extending from the caudal fin up to the pectoral fins[4].

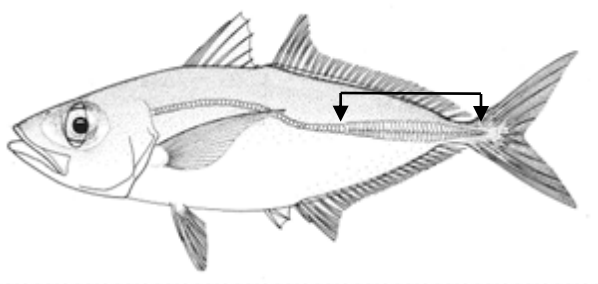


Fig. 5a. Diagrammatic representation of *Selar crumenophthalmus* showing the scutes extending half the whole body

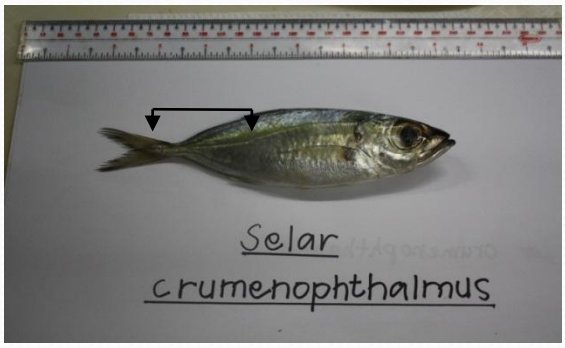


Fig. 5b. Actual picture of *Selar crumenophthalmus* showing the scutes extending half the whole body

Selar crumenophthalmus

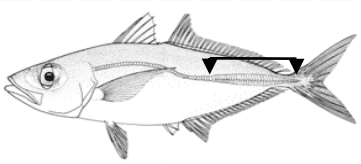
Class — Actinopterygii

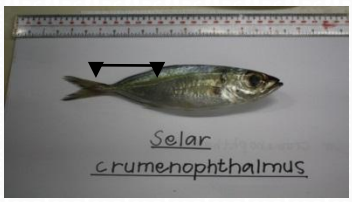
Order — others / Perciformes

Family — Lateral scutes absent: others / Lateral scutes present: Carangidae

Genus — deep furrow on lower margin of gill opening: *Selar* / others

Lateral scutes extending shortly from the caudal fin: ***Selar crumenophthalmus*** / Big lateral scutes extending from caudal to pectoral fin: *Selar boops*



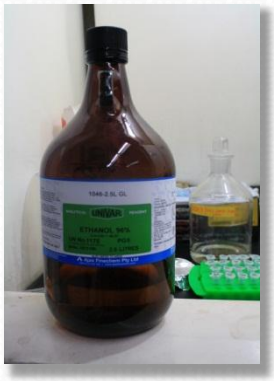


TISSUE SAMPLING

Tissue sampling requires that the tissue samples to be obtained are free of contaminants, otherwise, results of procedures following tissue sampling will be affected. This is especially important with studies involving subcellular analyses such as DNA and protein analyses.

Materials needed for proper tissue sampling are listed below:

70% ethanol



Absolute ethanol

bleach

1.5 ml microtubes

Forceps and scalpel

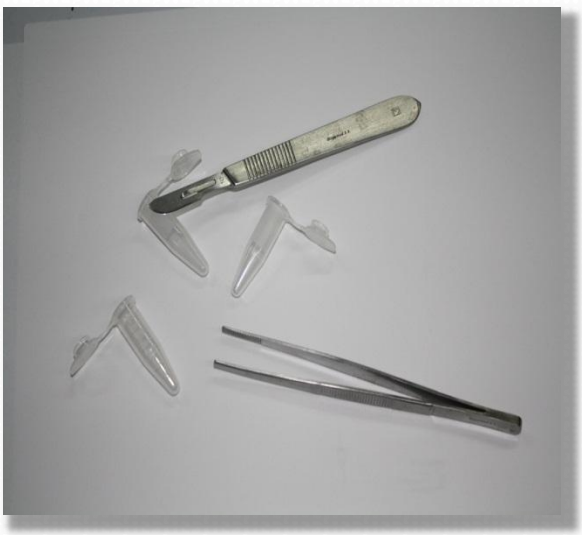
TISSUE SAMPLING

Clean the working area and dissection kit

It is always important to ensure that the working area and the dissection kit is not contaminated.

Wear clean gloves and clean the working area by spraying 70% ethanol and wiping it dry with a tissue or any clean, dry cloth.

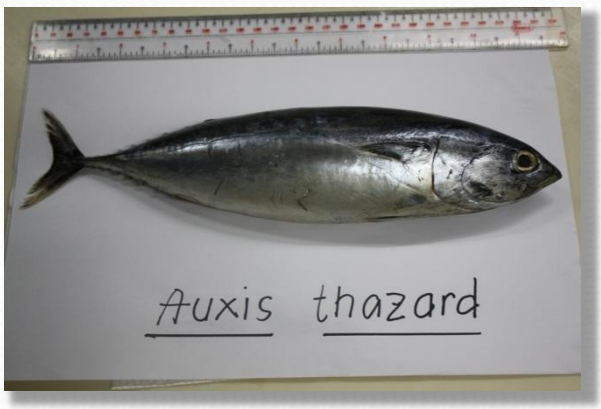
Use bleach and 70% ethanol to sterilize the dissection kit respectively. Always do this step after extracting one tissue from one kind of species. This is to avoid any contamination of tissue samples from one species to the other.

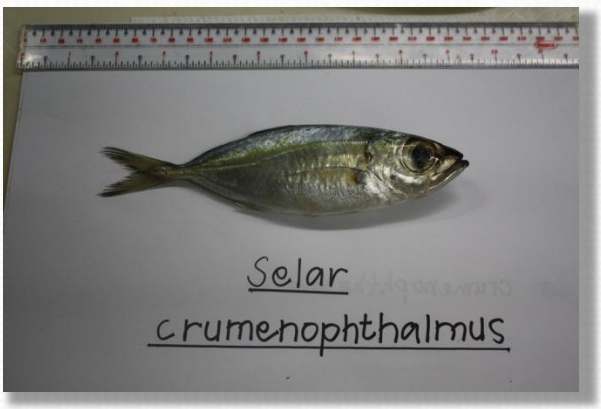


TISSUE SAMPLING

Documentation of the fish samples

Documentation is done by measuring the length (mm) and weight (g) of the fish samples and recording it in a table (table 1). Capture also an image of the fish samples. A ruler can be included in the picture taken so as to set a scale.





TISSUE SAMPLING

Table 1. Sample data blanks for sample documentation

No.	Sample name	Species	Length (cm)	Weight (g)

TISSUE SAMPLING

Make an incision and remove the skin using the scalpel

Make an incision and cut through the muscles of the fish at the left dorsal side. This is for standardization since the right side is used for documentation purposes. After making the incision, carefully separate the skin from the muscle. The muscle tissue is the one to be extracted and needed as a sample so the skin would not be necessary.

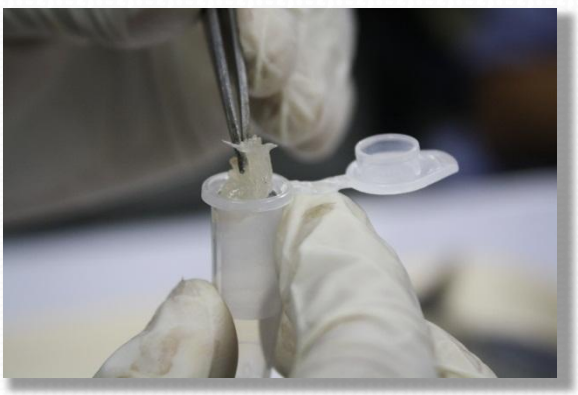
TISSUE SAMPLING

Remove the muscle tissue and put in the microtubes

Carefully remove the muscle tissue sample from the fish, making sure no skin are included in the sample. Make sure that a sufficient quantity of tissue has been collected.

Put the muscle tissue previously removed inside a 1.5ml microtube which contains absolute ethanol. Absolute ethanol preserves the sample just in case the sample is not yet to be used immediately.

The tubes with the samples inside are then stored in a refrigerator until use.



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