UMT MOOC
Massive Open Online Course

MINISTRY OF HIGHER EDUCATION

UMT

UMT MOOC
Massive Open Online Course

BIOLOGICAL CLASSIFICATION OF MARINE ORGANISMS

Preservation of Biological Collection

Part 1: Wet Collection

Azwarina Bt Mohd Azmi Ramasamy

Senior Curator

South China Sea Repository And Reference Center,

Institute Oceanography and Environment,

Universiti Malaysia Terengganu

What are wet collections?

- Wet collections are specimens kept in a liquid preservative to prevent their deterioration.
- Certain biological specimens are preserved in a wet form due to:
  - convenience
  - an intent to preserve body form and soft parts for a variety of uses
- When **color preservation is not critical** and dry preservation sacrifices qualities needed for other intended uses, fluid preservation is beneficial.

What is fixation?

- Fixation is a stabilization process in which the fixative chemically bonds to the specimen to impede deterioration by enzymatic digestion or autolysis.
- Formalin, a solution of 40% formaldehyde gas in water that is then further diluted, is a common fixative.
- Usually the final solution contains about 4% formaldehyde in water and is referred to as **10% formalin**.

Are all wet specimens treated with a fixative?

- Some specimens are **not treated with a fixative**, but instead are placed immediately in an **alcohol**.
- Alcohols replace water in the tissues to reduce the potential for deterioration.
- Alcohols are considered to be **denaturants**, rather than **fixatives**.

What types of preservative fluids are used for wet collections?

- **Preservative fluids** are those in which the specimen is housed for **long-term storage**, usually during the processing stage of specimen preparation.

- Alcohols, primarily **70-90% ethanol** and **50-60% isopropanol**, are common storage fluids for specimens that have been fixed or denatured.

What are the primary agents of deterioration for wet collections?

➔The primary causes of deterioration are usually related to **fluid and container quality**.

➔Other agents of deterioration include:

- Neglect
- Visible and UV light (light and ultraviolet radiation)
- Inappropriate temperature (temperature fluctuations)
- Inappropriate relative humidity and fluctuations
- Fire (especially for collections stored in alcohols)
- Physical forces
- Contaminants resulting from improper mixing of fluids

Fluid preservation

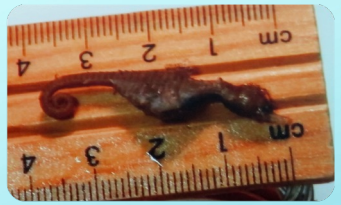
1) Defrosting
2) Rinsing
3) Assigning tags (or SRN)
4) Measurement
5) Obtaining tissue for DNA analysis
6) Spread fin
7) Pre-fixation
8) Photography
9) Tagging
10) Identification
11) Formalin fixation
12) Rinsing
13) Replace to alcohol
14) Specimen labels
15) Storage and seal

Fluid preservation flow chart

1. **Defrosting**

2. **Rinsing**

3. **Assigning tag**



4. **Measuring**

5. **Obtaining tissue**

6. **Spreading fin**

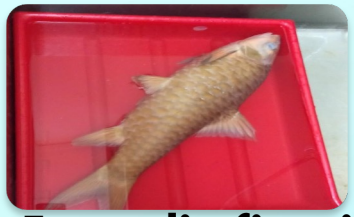
7. **Pre-fixing**

8. **Capturing photo**

9. **Tagging**

Fluid preservation flow chart

10. **Identification**



11. **Formalin fixation**

12. **Rinsing**

13. **Replace to Alcohol**

14. **Label**

15. **Storage**

Sampling

Collecting samples by team

Pack with marine water and label sampling information

Prevent freeze-burn

freezer -20˚C

Defrosting

First step in preparing specimens from fish samples frozen for a long time.
Should be initiated at the right time.
Should be initiated considering the room temperature and the mass of the ice supplied with the samples.
Started too early, the fishes will begin decaying, esp. the internal organs.
On the other hand, if thawing is delayed, sufficient ice will not melt.
In this situation, however, the remaining unmelted ice should not be pulled from the fish bodies, since it will break the scales and surface skin.
In addition, scrupulous attention is required while thawing a few frozen samples together, to avoid mixing up of data notes.

Defrosting under running water.

Rinsing

- Make sure rinse the sample with clean water to remove any mucus, dirt and any unwanted items.
- After that, put the sample in the **black rubber dissecting tray** and support with **white polystyrene/ foam.**

Before and after rinsing

Assign tag

- Find tag number or specimen reference number (SRN).
- Assign for the sample and record in the datasheet provided.

Measurement

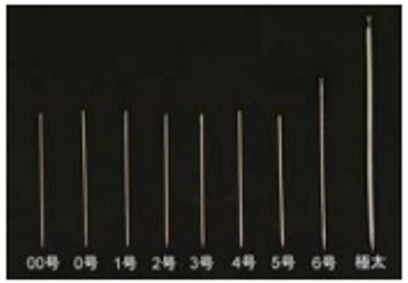
Fish Measuring Board

- ✓ Take 3 measurements of the fish in **millimeter (mm)** (standard length, total length and fork length) using fish measuring board.
- ✓ After that, weight the sample in **gram (g) using** top pan balance.
- ✓ Records all data in the datasheet provided.

Caliper used in the Kagoshima University Museum. Above: VC-30, M-type standard caliper by Mitutoyo, Japan. Below: CD-SC, series 500 ABS digimatic solar caliper by Mitutoyo, Japan.

Spread fin and pre-fix

Spread fin and pre-fix



Entomology pin

Polystyrene of foam

Brush

Laboratory tissue

Water spray

10% formalin

Spread fin

- Position sample in horizontal, **head on the left**.

- Take 4 big pins place at head area. Pin at upper side and downside. Then, pin at the tail/caudal area upper side and downside. As picture showed close the mouth and make sure the body straight.

- Entomology pin used to fix the position

1. Fixed body axis.

2. Spread and fixed caudal fin.

3. Spread and fixed dorsal fin ray from the rear.

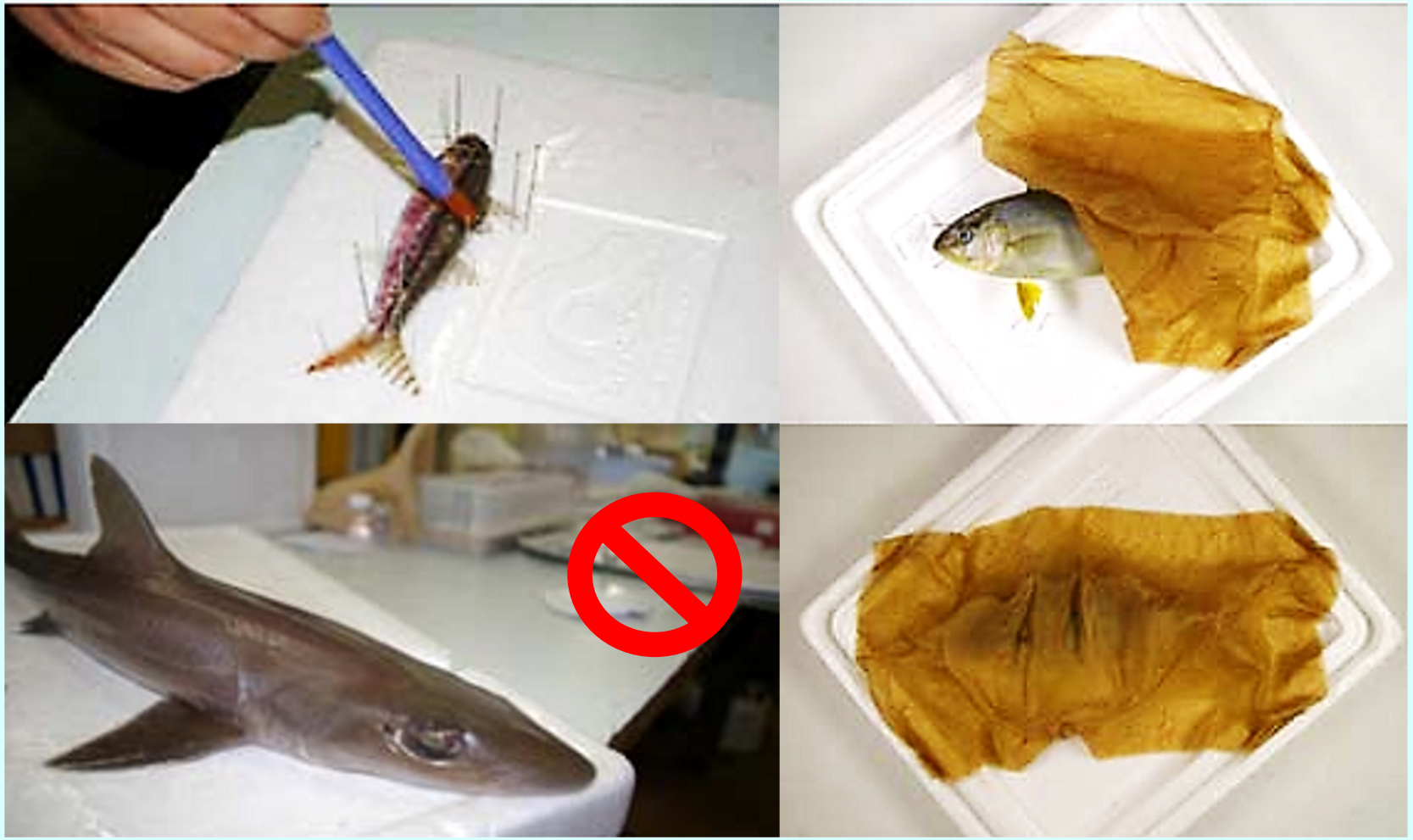
4. Spread and fixed anal fin.

5. Spread and fixed dorsal spiny ray.

6. Spread and fixed pelvic fin.

Process of fin spreading for specimen of *Seriola dumerili*.

Pre-fix



No need pre-fix

Cover with wet tissue

Photography

Close-up copy stand, light, and glass aquarium for small specimens.

Close-up copy stand, light, and glass aquarium for medium specimens.

Glass aquarium should be placed a little above the white board on the close-up copy stand. Here, square wooden blocks are used as legs for the aquarium.

Specimen is stably lying on the bottom of the water-filled aquarium. Above: view from the back. Below: view from the front.

A slight tilt in the fish position can be adjusted using a rubber piece (*e.g.*, an eraser piece).

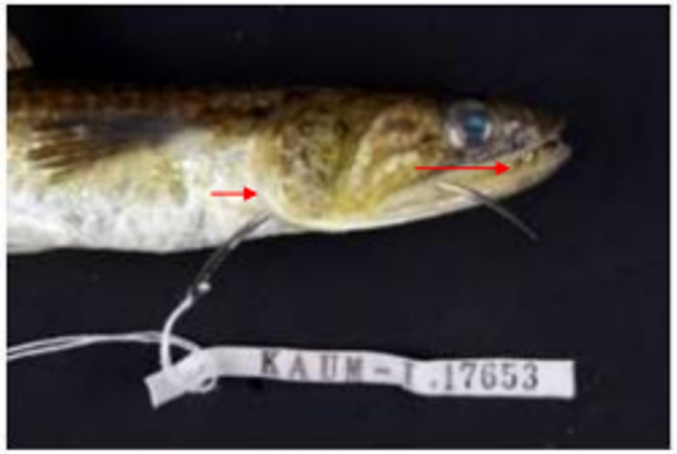
Tagging

White cotton thread

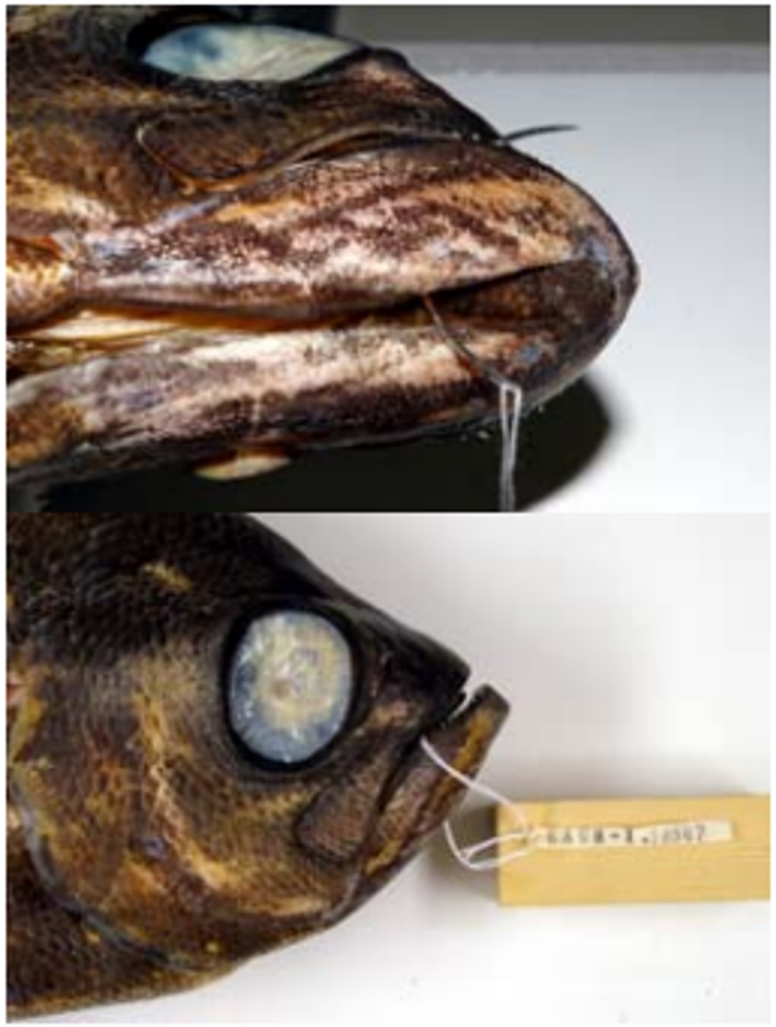
Stainless steel forceps

Specimen Reference Number / Tagging number

- Cut a small amount of thread around 10cm long.
- Using forceps bring the thread from behind the operculum through the gill slit and out from mouth.
- Then, put the tag and tie the thread.
- Leave 1 finger space/hole between the tag and sample.



Typical method of tagging. The threaded needle is passed through the right gill slit to the mouth.



Tagging through the lower jaw. The soft tissue between dentaries should be pierced.

For tagging sharks and rays, the base of the right pelvic fin should be pierced.

For tagging members of Syngnathidae, tie the thread at the sulcus of arthromeres.

Identification

- Using the information provided search the common name of the sample in the internet to get the nearest or possible scientific name.
- Verify the scientific name using WORMS portal. https://www.marinespecies.org/index.php
- Find accepted scientific name and others info about the species such as Order and Family.
- Record the info in the datasheet and label of the sample.

PUSAT RUJUKAN & REPOSITORI LAUT CHINA SELATAN
UNIVERSITI MALAYSIA TERENGGANU (UMT)

Order : Tetraodontiformes **Family**: TETRAODONTIDAE
Species : *Chelonodon patoca* (Milkspotted puffer)

Specimen Ref. No.: UMTF 0077	**Collected on :** 16-30/08/07	**Collected by :** Siti Tafzil/ Shukriah
Locality and remark : Setiu Wetland (Pulau Semut)		**Identified by :** Siti Tafzil

Fixation

10 % formalin

1 : 9

Formalin : Distill water

For Invertebrate

1. Fix in ethanol 75-80% without fix in formalin.

Rinsing and replace to ethanol

Remove formalin by pour it into chemical waste bottle and rinse the sample with tap water

After remove from tap water, immerse the sample in 75% ethanol for stabilization in long time.

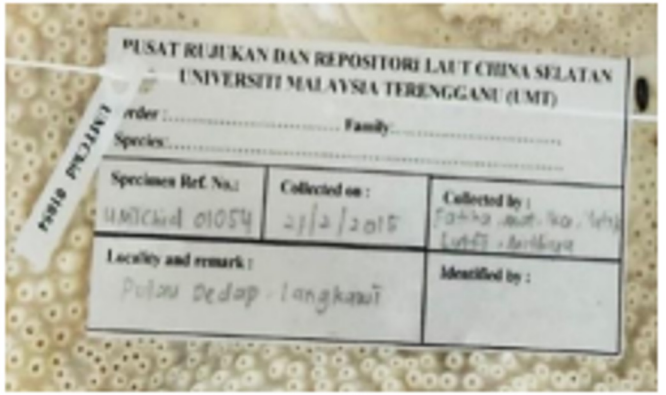
Labelling

PUSAT RUJUKAN & REPOSITORI LAUT CHINA SELATAN
UNIVERSITI MALAYSIA TERENGGANU (UMT)

Order : Tetraodontiformes **Family**: TETRAODONTIDAE

Species : *Chelonodon patoca* (Milkspotted puffer)

Specimen Ref. No.: UMTF 0077	**Collected on :** 16-30/08/07	**Collected by :** Siti Tafzil/ Shukriah
Locality and remark : Setiu Wetland (Pulau Semut)		**Identified by :** Siti Tafzil



Use **ONLY** carbon-based, black inks on specimen labels such as carbon pencil. Carbon inks do not fade over time in alcohol.

After transfer and write all information into label, using forceps put the label into the specimen bottle/jar.

Storage

- Best storage is **specimen jar** or **glass specimen bottle with black phenolic cap**.
- Please choose one bottle, best to suit your specimen. Put your specimen and pour enough ethanol inside it.
- Make sure the ratio between specimen and ethanol is **40:60**.
- More alcohol than the specimen volume to make sure the specimen not deteriorate.

Seal

- Best seal is glass specimen bottle with **black phenolic cap** because it come with foam inside the cap to tighten the bottle.
- After tighten your specimen bottle. Seal the cap to reduce ethanol evaporation.
- Use laboratory sealing film such as PARAFILM to seal around the cap.

MINISTRY OF HIGHER EDUCATION

UMT

UMT MOOC
Massive Open Online Course

Thank You