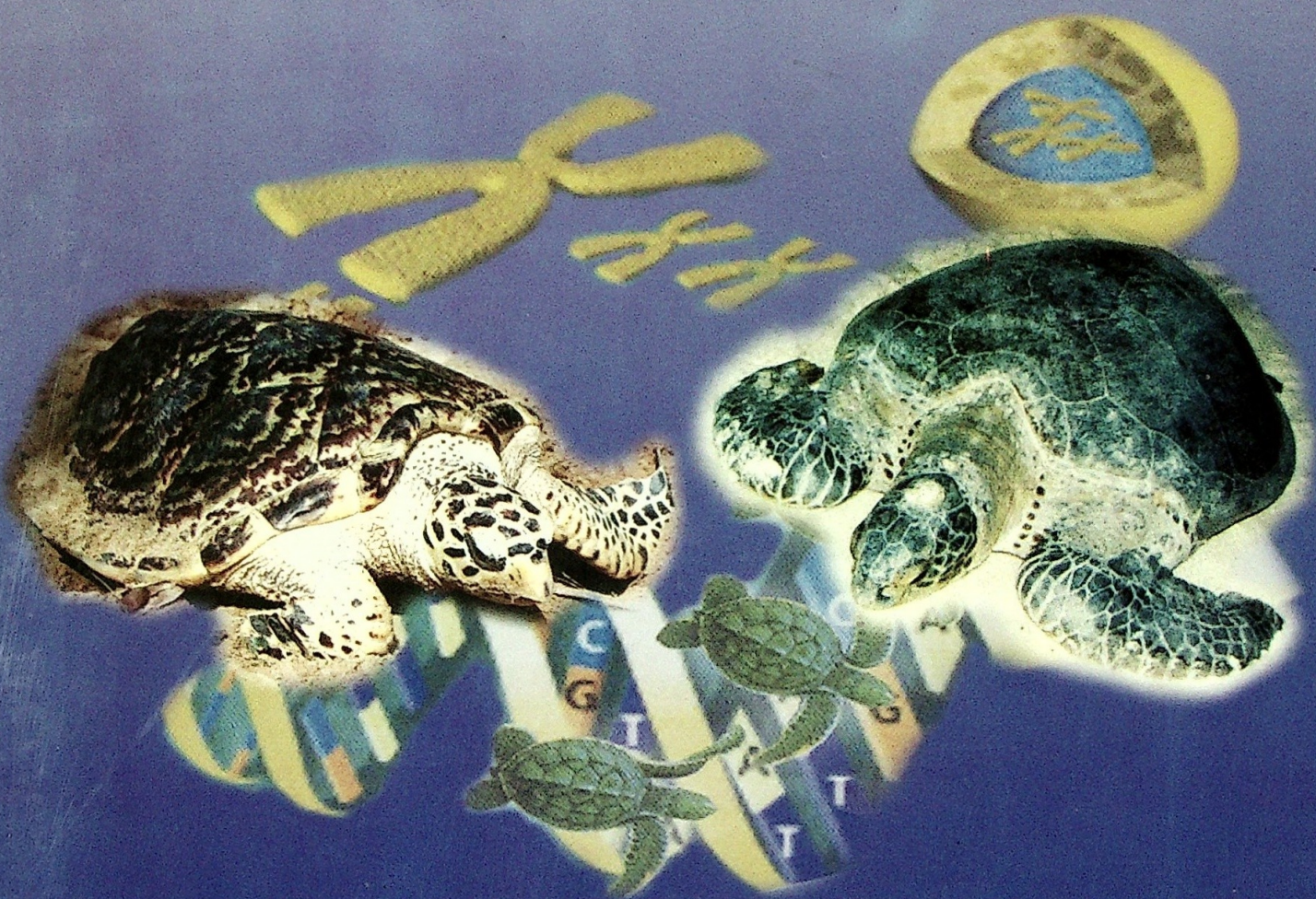




STANDARD OPERATING PROCEDURE

Sampling Tissue of Sea Turtles in the Southeast Asian Region



DATE :
BAR CODE :
ACCESSION NO :

Sampling Tissue of Sea Turtles in the Southeast Asian Region

Identification the Stock Population of Sea Turtles

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Standard Operating Procedure (SOP) - Sampling Tissue of Sea Turtles in the Southeast Asian Region

Introduction

Sea turtles are highly migratory and share the waters of Southeast Asian region. Within a region, conservation efforts to protect sea turtles in one country may be jeopardized by irresponsible activities in another country. Thus regional cooperation among member countries in conserving sea turtles is vital to ensure their survival. Understanding the discreteness of sea turtle population or sub population and how the populations relate to each other is crucial for proper regional management of these endangered species. A management approach through understanding the stock population or management unit is therefore highly recommended.

In this regard, the Regional Technical Consultation on Finalization of Implementation Plan on Research for Stock Enhancement of Sea Turtles the SEAFDEC member countries had agreed to conduct the study on population genetics of Green Turtle (*Chelonia mydas*) and Hawksbill Turtles (*Eretmochelys imbricata*) in the Southeast Asian region. Twenty rookeries have been selected for the collection of the tissues. This covering the entire Southeast Asian Countries except for the Laos and Singapore. As for Green Turtles the tissue samples collecting and analyzing will be started from 2005 until 2006, while for Hawksbill Turtles for 2006 until 2008.

Target species:

Green Turtles (*Chelonia mydas*) and Hawksbill Turtles (*Eretmochelys imbricata*)

Objectives:

This SOP will provide a standardized method of sampling tissues of adult sea turtles; *Chelonia mydas* and *Eretmochelys imbricata* during the nesting activities in selected rookeries of the Southeast Asian region. This SOP will ensure the samples are not contaminated thus providing reliable and accurate data.

Sampling Sites

The Selected Sites are:

Brunei beach (**Brunei Darussalam**), Sihanoukville (**Cambodia**), Derawan, Pangubahan and Segamat (**Indonesia**), Mak Kepit, Segari, Sarawak Turtle Islands and Sabah Turtle Islands (**Malaysia**), Thamee Hla Island, Laung Lon Bok Island (**Myanmar**), Northern Sierra Madre, Apo Reef Natural Park, Panikian Islands, Philippines Turtle Islands (**The Philippines**), Khram Island, Huyong Island (**Thailand**), Minh Chau Island and Con Dao Island (**Vietnam**).





Figure 1: The selected rookeries for collection tissue sample of Green Turtles (*Chelonia mydas*) in the Southeast Asian region

Number of samples

Tissues from at least thirty (30) individuals of different adult female turtles are required. If the landings are not frequent, not less than ten (10) individuals will be needed.

Size of tissues

The size of tissues samples should approximately one centimeter square (1cm²).

Duration on collecting samples

For Green Turtle the sampling should start in 2005 and end in April 2006 and for Hawksbill Turtle, for 2006 till April 2007.

Number of workers

Minimum two or three persons are required for the sampling activities: One person will hold the turtle from moving, one person to trail the hind flipper and one person to grip the soft muscle with forceps and to cut it with scissor.

When to take the samples

Tissue sampling activities should be conducted after the turtles have completed their nestings.

Tissue Sampling Protocol



Equipment Required

1. The equipment that are required for tissue samplings of sea turtles are; screw vial, vial container, ethanol or buffer solution, distilled water, betadine, latex gloves, permanent marker, labeling, scotch tape, labeling, washing bottle, tissue and lighter.



Procedures / Instruction

1. Every sampler should wear gloves. Wash the forceps with clean water.



2. Wipe the forceps with soft tissue paper.



3. Wash the forceps using ethanol solution with 90 - 100% concentration.



4. Wipe the forceps again with soft tissue paper.



5. Wash the forceps again with ethanol solution with 90 - 100% concentration.



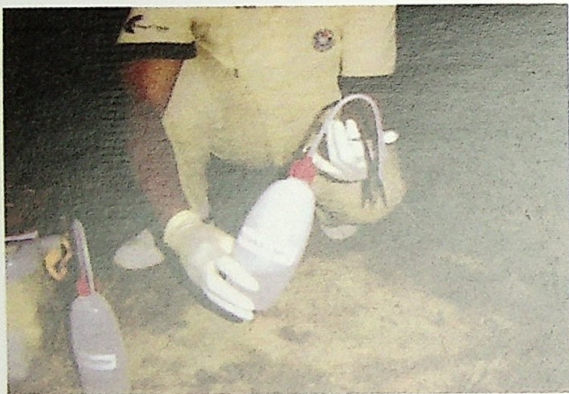
6. Sterilize the forceps using a lighter before the ethanol evaporates.



7. Then wash the scissor with clean water.



8. Wipe the scissor with soft tissue paper.



9. Wash the scissor using ethanol solution with 90 - 100% concentration.



10. Wipe the scissor again with soft tissue paper.



11. Wash the scissor again using ethanol solution with 90 - 100% concentration.



12. Sterilize the scissor using a lighter before ethanol evaporates.



13. Collect the tissue after the sea turtles have completely laid their eggs. One person / worker should hold the turtle before she goes to sea.



14. Carefully hold the sea turtle while another person position at the back for collecting tissue.



15. Trail the hind flipper and hold the soft muscle.



16. Wipe any sand off the ventral surface of the rear flipper 5 - 10 cm from the posterior edge with tissue paper.



17. Clean the ventral surface of the rear flipper with ethanol solution using soft tissue paper.



18. Hold the soft muscle with the forceps and quickly cut the tissue with the scissor. The size of tissue should be about 1.0 cm².



19. Hold the tissue with forceps carefully and clean it with ethanol solution.



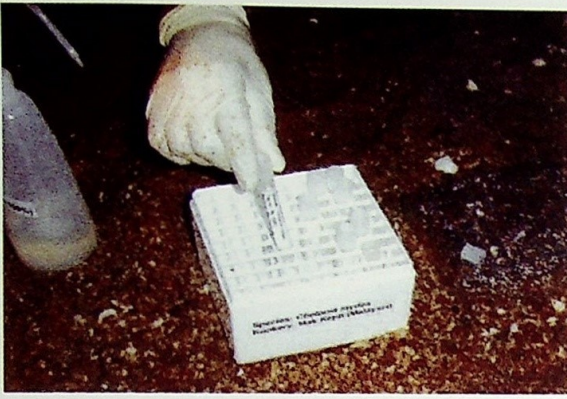
20. Transfer tissue into screw vial.



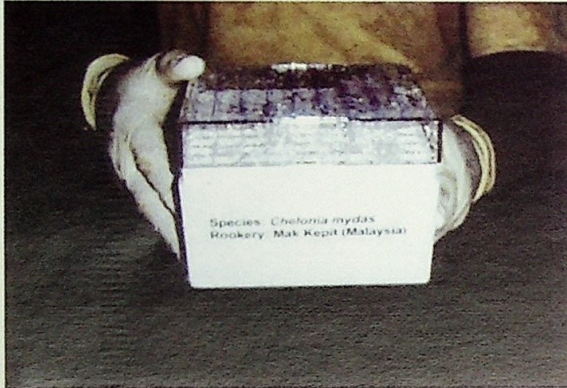
21. Add ethanol solution with 90 - 100% concentration or buffer solution (NaCl with 20% DMSO) into screw vial for tissue preservation. The ratio of ethanol or buffer solution is, 1 part of tissue : 7 to 10 of ethanol / buffer solution.



22. Label the vial accordingly by **number, species, tag number, rookery and date of sampling** and cover the label with scotch tape. This will ensure that the label will intact longer.



23. Place the label screw vial into the vial container according to the vial number.



24. Label the vial container accordingly by *species and rookery*.



25. Take a few milliliters (ml) of liquid betadine with pipette dropper.



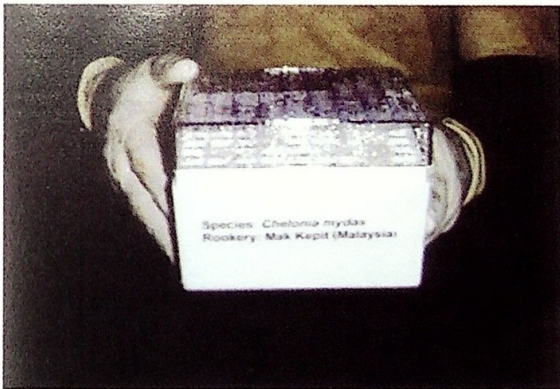
26. Drop a few milliliters (ml) of betadine to soft cotton.



27. Swipe the betadine to the injured muscle of sea turtles.



28. Make sure that sampled sea turtles were tagged and the particular tag number should be recorded for the tissue samples.



29. Place the vial container which consist tissue samples in a refrigerator or freezer immediately after completing sampling activities with minimum temperature value of - 4°C.

30. Key in the data of tissue sample into a computer using Excel softwear.

Example on tissue samples data sheet

No.	Species	Data	Rookery	Tag No.
1	<i>Chelonia mydas</i>	15.06.05	Selingan Island Sabah	MY(s) 29831 / MY(s) 29552
2	<i>Chelonia mydas</i>	15.06.05	Selingan Island Sabah	MY(s) 29843 / MY(s) 29844
3	<i>Chelonia mydas</i>	15.06.05	Selingan Island Sabah	MY(s) 27555 / MY(s) 27556
4	<i>Chelonia mydas</i>	15.06.05	Selingan Island Sabah	MY(s) 29717 / MY(s) 29718

Finally, sent the tissue samples of sea turtles together with sampling data to the following address:

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Taman Perikanan Chendering,
21080 Kuala Terengganu, Malaysia.
With attention to:
Mr. Syed Abdullah Syed Abd. Kadir and
Ms. Wahidah Mohd. Arshaad.*

References

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