

CHAPTER 2

MATERIALS AND METHODS

I. Preliminary Survey

Preliminary surveys, by means of questionnaires (Appendix 1), were carried out in Chon Buri, Samut Sakorn, and Phetchaburi Provinces. Target species at these locations are *Rhopilema hispidum* and *Lobonema smithii*. At Chon Buri Province, jellyfish fishery starts from April until October. At Samut Sakorn Province, jellyfish fishery generally starts in April until the end of the year. At Phetchaburi Province, fishery is conducted in July and August depending on the meteorological conditions of each year. The fishing methods used in all three provinces appeared to be dip nets. However, jellyfish are also obtained as by-catch from boats equipped with push nets (Table 5).

Table 5. Summary of interviews carried out in Chon Buri, Samutsakorn, Samut Songkram, and Phetchaburi Provinces.

	Province			
	Chon Buri	Samutsakorn	Samut Songkram	Phetchaburi
Fishing Season	April, June - October	April-December	April, September-December	July-August
Fishing Equipment	Dip nets	Dip nets, push nets	Dip nets, push nets	Dip nets
Fishing Time	Day and night	Day	Day and night	Day and night
Fishing Areas	Sriracha, Laem Chabung	Tachin River's mouth	Maeklong River's mouth	Along the coast, Baan Laem
Target Species	<i>Lobonema smithii</i> and <i>Rhopilema</i> sp.	<i>Lobonema smithii</i> and <i>Rhopilema</i> sp.	<i>Lobonema smithii</i> and <i>Rhopilema</i> sp.	<i>Lobonema smithii</i> and <i>Rhopilema</i> sp.

Aboard the long-tail boats, fishermen visually locate swarms of jellyfish. Jellyfish are then fished out of the water with the dip nets, with the mesh size of approximately 1 cm, on to the floor of the boat. According to Cornelius (1995), jellyfish sampling is accomplished by means of an angler's

landing net, which is suitable for collecting small to medium size specimen. This method, however, is rather insufficient and impractical in the case of systematic sampling.

Subsequent to a preliminary survey, through means of questionnaires and interviews, it was decided that samples would be collected off the coasts of Baan Laem, Phetchaburi and, south of the Bangpakong River's mouth, Chon Buri Provinces. Both locations are situated in the Inner Gulf of Thailand, approximately opposite each other, and jellyfish fishery is common practice.

II. Scyphomedusae Sampling

A. Collection

A fishing boat equipped with a push net (mesh size ≈ 0.5 inch, mouth width ≈ 12 meters), operating at an approximated speed of 3 to 4 km/hr, was chosen for quantitative jellyfish sampling (Figure 8). The sampling procedures are as follows:



Figure 8. Sampling boat equipped with push net.

1. At each location, a total of three sampling lines running parallel to each other, at approximately half a kilometer apart and perpendicular to the shoreline were established. The length of each line was measured by a time unit, which was trawling time of 10 minutes (Figure 9).
2. After each ten-minute trawl, or at the end of each sampling line, the net and its contents were retrieved. All medusae were isolated from the rest of the contents. Medusae obtained from each sampling line were stored separately.
3. Sampling was conducted once a month, during daytime spring tide, for a period of 13 months from December 1999 to December 2000.



Figure 9. Map of sampling areas.

B. Fixation and Preservation

1. The fixation solution recommended by UNESCO (1976) is 10% formalin (buffered). Borax (Sodium Tetraborate Decahydrate) buffered formalin was prepared as follows:

30 g. of borax per 1 liter of concentrated formalin

The buffered solution was diluted by the addition of seawater in a 1:9 ratio (i.e. 10 ml of concentrated formalin to 90 ml of seawater) to yield 10% formalin fixative.

2. After 7 to 10 days, specimens were transferred into a preserving solution. In the case of coelenterates, 70% ethanol is a better alternative compared to formalin (Omori and Ikeda, 1984). To prevent an immediate loss of water, specimens were first transferred to 30% ethanol to be preserved for approximately one week prior to transfer to 70% ethanol.
3. All specimens obtained from the trawls were fixed and transported back to the laboratory. If the amount of specimens obtained was extremely large (> 200 individuals per haul), each specimen was measured for weight and bell diameter. Representatives of the specimens were selected, based on physical resemblance, and fixed prior to transportation to the laboratory.
4. Plastic containers are used for temporary storage of specimens. Long-term storage in polyethylene container causes dissolved complex of paraffin and softening agents to settle as fine, film coating on specimens. This will obscure taxonomically important details necessary for identification purposes. For safety reasons, plastic containers are generally used on board. However, specimens must be transferred to glass containers once they arrive at the laboratory.

C. Identification

All medusae obtained from the field were measured for weight and bell diameter and identified. Identification of specimens is accomplished using existing taxonomical keys i.e. Keys to Thai and Malaysian Cubomedusae and Scyphomedusae (Cornelius, 1995). Although the structures of nematocysts can be used for identification, only external characteristics were used in this study. The physical characteristics that were important to the identification process were shape of bell, exumbrella surface, color patches, presence of terminal filaments or clubs, and mouth arms (Figure 10). Most distinguishing characteristics were recognizable under the naked eyes. However, inconspicuous features, such as the terminal filaments, were best viewed under a stereomicroscope.

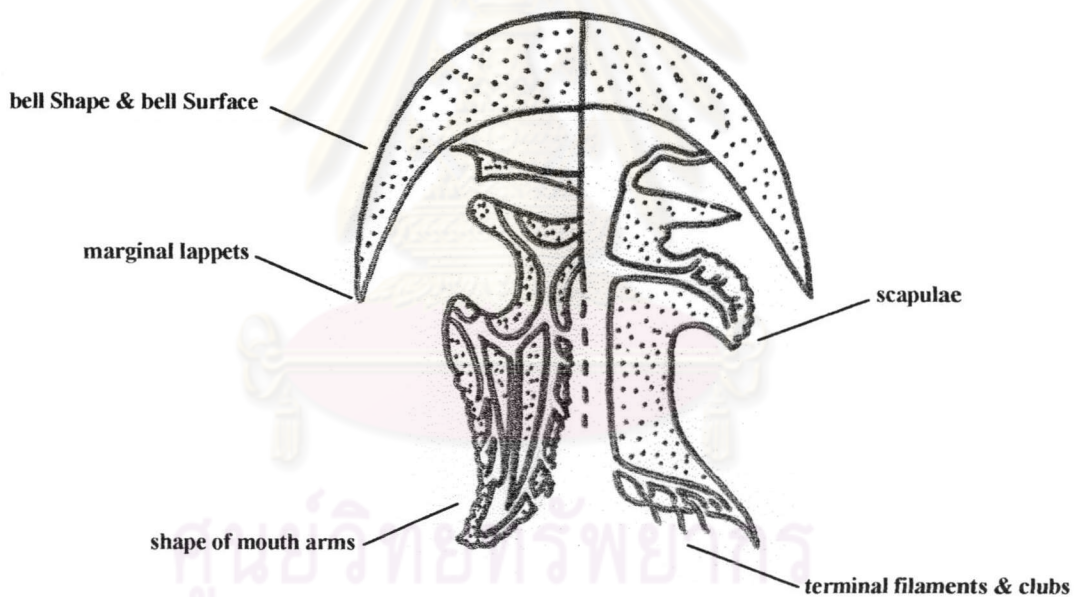


Figure 10. Physical characteristics used in the identification of scyphomedusae (Mianzan and Cornelius, 1999).

D. Abundance

Abundance of rhizomedusae, or number of individuals per 10,000 m³ of water, was calculated using the following formula:

Trawling distance (m) = Speed x Time

Speed = Speed of the boat (km/h), which was recorded using the GPS

Time = Trawling time (hr)

Thereafter, the volume of water trawled was calculated by:

Volume of water (m³) = Length x Width x Depth

Length = Trawling distance (m)

Width = Width of push net's mouth (m)

Depth = Depth of water (m)

The average abundance for each month, at each location, was calculated from the abundance obtained from each sampling line. In addition to the average abundance of rhizomedusae, specimens were categorized into various size classes using the measurements of the bell diameter.

E. Gonad Analysis

1. For each species of rhizomedusae obtained from sampling, specimens of varying sizes were selected for gonad analysis.
2. The gonad of each selected specimen was extracted and smeared onto a slide using a scalper. After adding a small amount of water and placing the cover slid on top, the sample was pressed, by lightly tapping on the cover slid, so that it was evenly distributed on the slide.
3. Samples were viewed under the compound microscope and pictures of each sample were taken.

III. Zooplankton Sampling

A. Collection

At the beginning and the end of each ten-minute sampling, vertical zooplankton samplings were conducted using the standard zooplankton sampling net with the mesh size of 330 μm .

B. Fixation and Preservation

Zooplankton samples were immediately fixed in 4% borax buffered formalin solution and transported back to the laboratory.

C. Identification

Each zooplankton sample was counted and identified into major groups, i.e. copepods, fish larvae, amphipods etc., under a stereomicroscope.

D. Abundance

Zooplankton abundance, or number of individuals $\cdot 10^2 \text{ m}^{-3}$ of water, for each month, at each location, was calculated by means of readings of the flow meter using the following formula,

$$T = \frac{100 \times t}{V}$$

T = Abundance of zooplankton (individuals $\cdot 10^2 \text{ m}^{-3}$ of water)

t = Actual number of individuals counted from sample

V = Volume of water filtered by zooplankton net (m^3)

V was calculated using the following formula,

$$V = \frac{a \times n}{N}$$

N

a = Area of mouth of zooplankton net

n = Actual flowmeter reading

N = Flowmeter calibration value

IV. Physical Parameters (Figure 11)

1. Few other parameters that were measured include visibility (secchi disk method), depth (using weight tied to a rope), salinity (SCT Meter), temperature (SCT Meter), dissolved oxygen (DO Meter), and pH (pH Meter).
2. Upon collection, on-site records included line number, geographical coordinates of the starting location and terminating location of each sampling line, trawling speed, trawling depth, dimensions of net, and notes on the atmospheric and ocean conditions.
3. All measurements of physical parameters obtained from each month (dissolved oxygen, temperature, and pH) were calculated for average and standard deviation.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

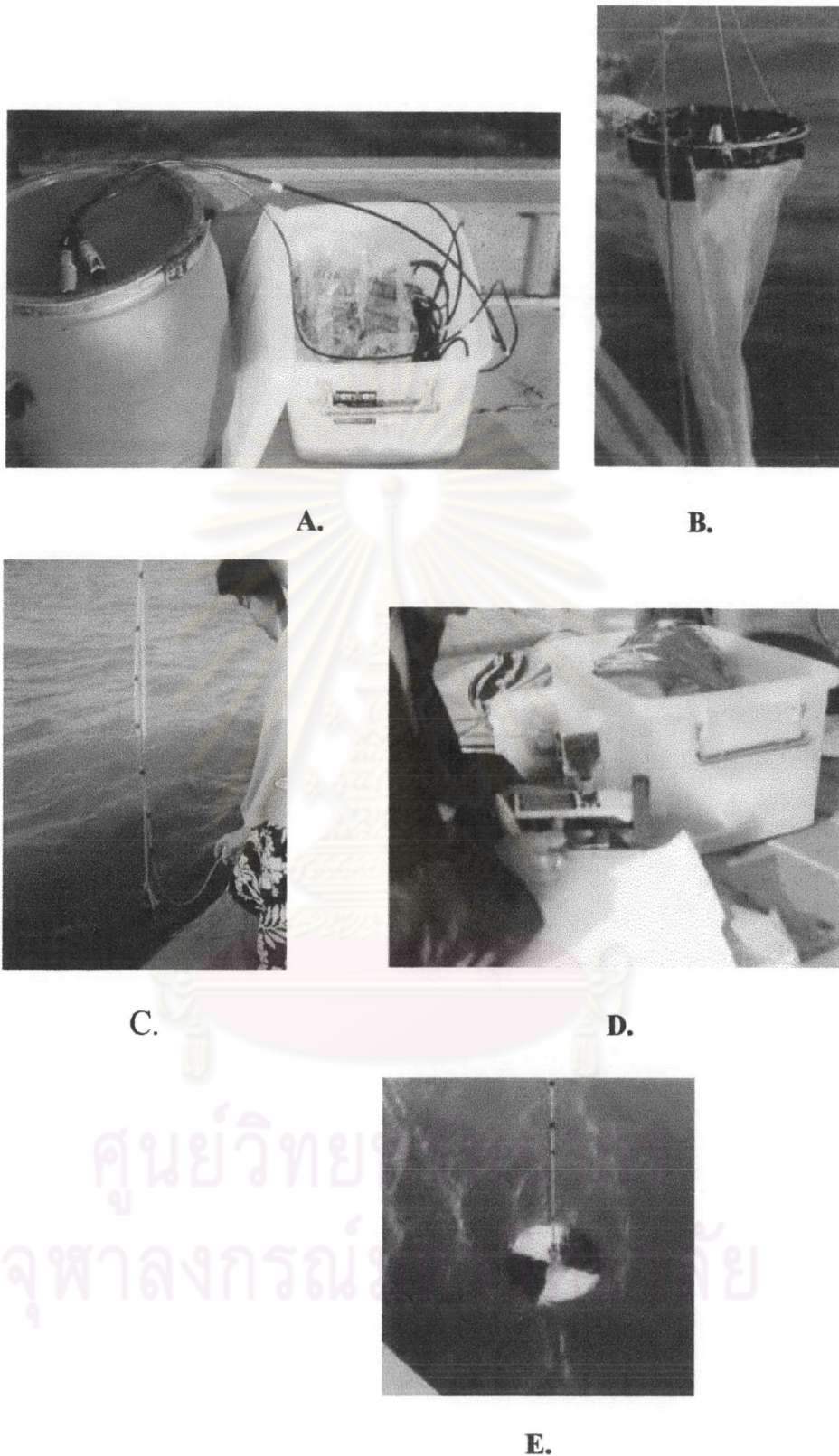


Figure 11. Sampling gears/equipment for physical and biological environmental parameter: (A) SCT Meter and DO Meter; (B) zooplankton net equipped with a flow meter; (C) depth measurement; (D) GPS; and (E) Secchi disc for measurement of transparency.