Sexual Reproduction of Millepora intricata and Millepora tenella (Hydrozoa : Milleporidae)

2. MATERIALS AND METHODS

2 - 1 Study site

Sampling of *Millepora* branches for histological examination was undertaken at the reef flat in front of The Tropical Biosphere Research Center (TBRC), University of the Ryukyus, which is located on the southeastern shore of Sesoko Island, Okinawa (26°38'N, 127°52'E) (Figure 3). The distance from the shore to the reef edge is approximately 80 - 100 m. The shallow reef flat is about 1 - 2 m deep at low water datum (LWD) (Figure 4). In 1997, the reef substratum was mainly carbonate and sand. Hard coral cover was approximately 8 - 10 % and soft coral cover was approximately the same. *Favia, Porites* and *Millepora* were the most common hard corals.

2 - 2 Field sampling

I chose 5 healthy colonies of *Millepora tenella* and 5 healthy colonies of *M. intricata* for my study undertaken between October 1996 and October 1997. I marked the colonies with nails and a small sheet of water proof paper, on which a colony number was written. Colonies of *M. intricata* were mainly distributed on the reef flat. On the other hand, *M. tenella* was less abundant, and was mainly found on the reef edge (Figure 4). All *M.*

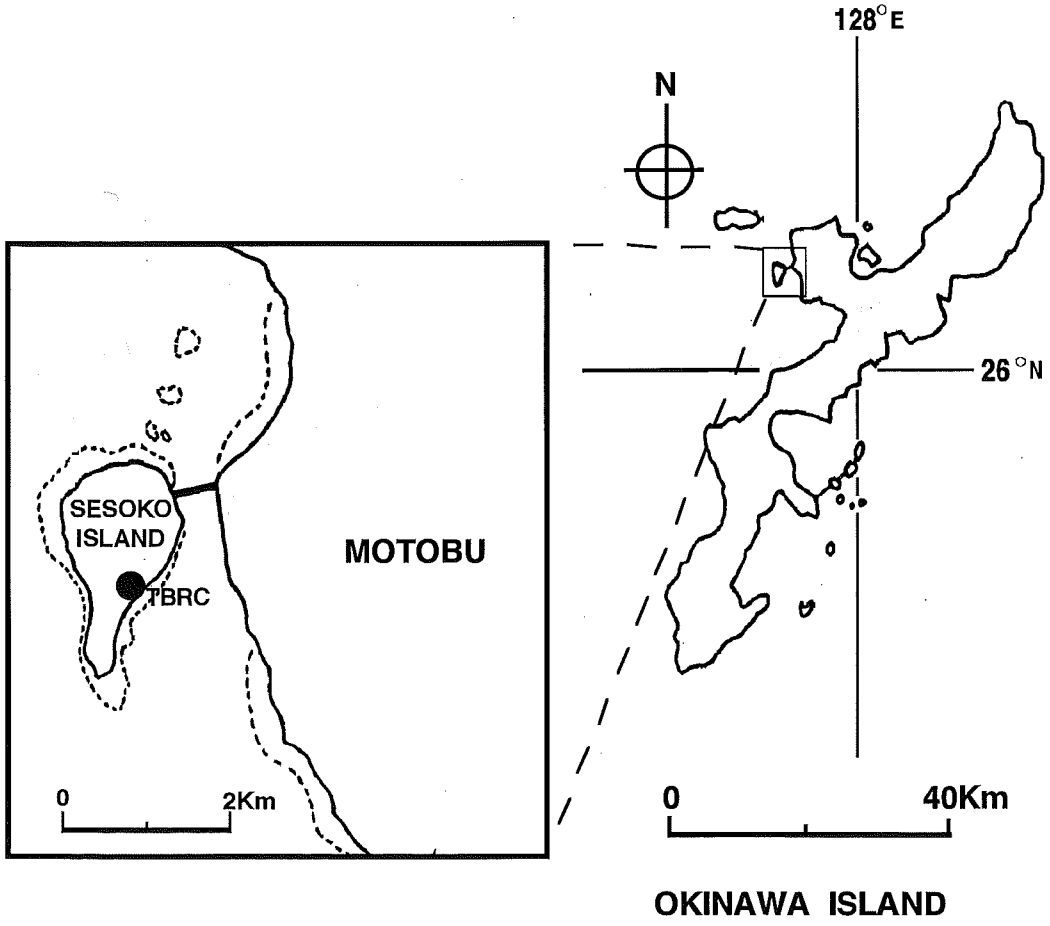


Figure 3. The sampling site, the coral reef in front of TBRC (Tropical Biosphere Research Center, University of the Ryukyus) located at Sesoko Island, Okinawa.

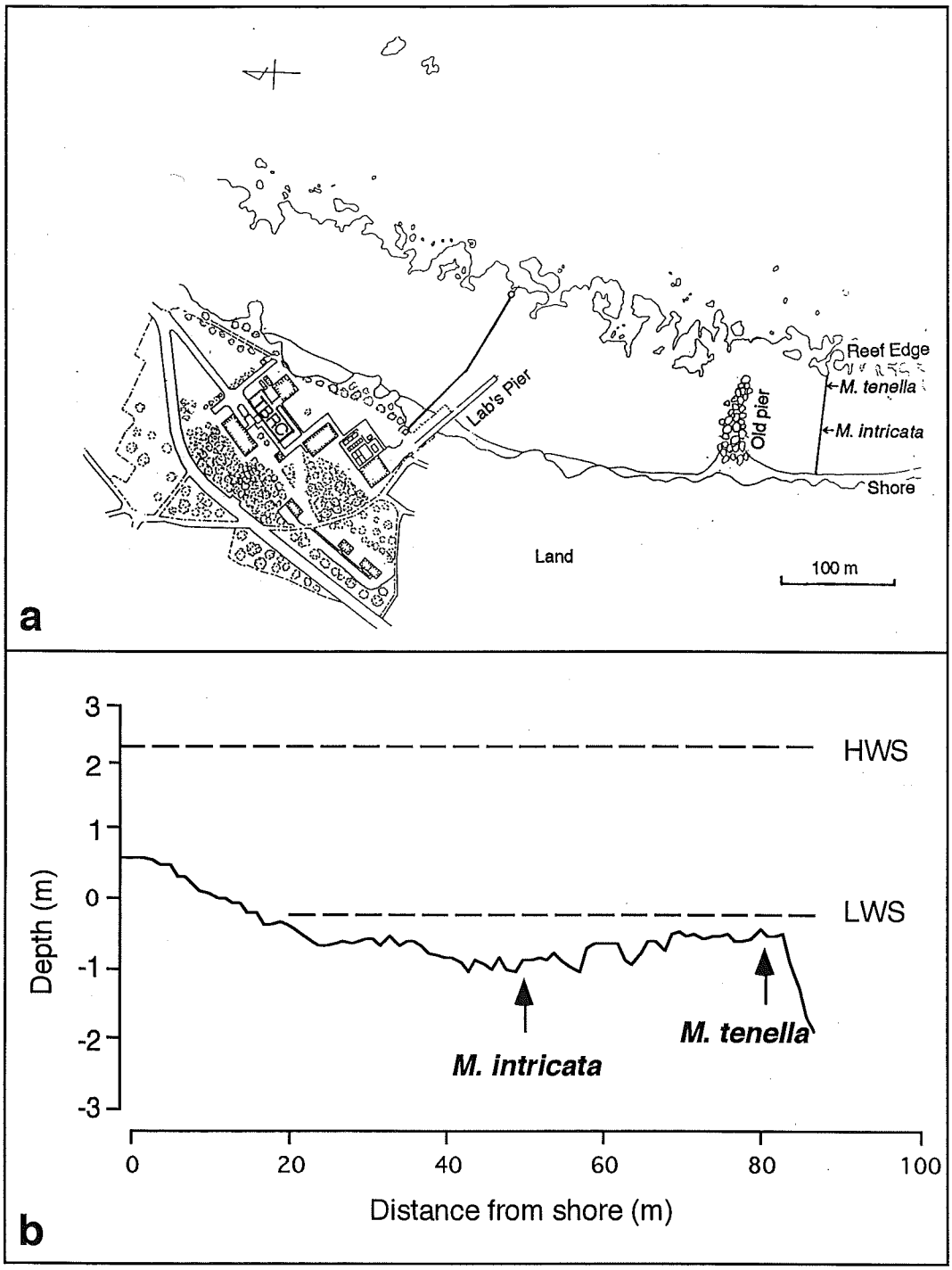


Figure 4. The sampling site. **a)** The coral reef in front of TBRC (Tropical Biosphere Research Center). **b)** A cross section of the reef. The arrows indicate the places where the marked colonies were located. Where HWS and LWS are high water spring and low water spring respectively.

intricata colonies had fine branching, dome-like, morphologies (Figure 1). *M. tenella* had large hand-like branches and encrusting bases (Figure 2).

Sampling was conducted once a month, from October 1996 to April 1997. The frequency of sampling was increased to once a week from May 1997, when ampullae began to appear, to September 1997. For each sampling period I collected one 6 - 7 cm branch from each marked colony. To consider the influence of light intensity, I took branches from both the top and the base of *M. intricata* colonies. In the case of *M. tenella*, the colonies are composed of an encrusting base and upright branches. Therefore, I sampled one 5 - 6 cm branch from the top of each colony.

2 - 3 Histological examination

The extracted branches were immediately fixed in 10 % formalin in seawater and then decalcified in 20 % acetic acid solution for about three days. The soft tissue, 2 - 3 cm from the tip of each branch, was cut into 0.25 cm^2 pieces. The growing tip was not analyzed because it may have been immature, as in the case of scleractinian corals. The soft tissue was dehydrated through a series of increased concentrations of ethyl alcohol (80 %, 95 %, 99 %) and Benzene, and then embedded in paraffin wax

(m.p. 56 - 54, Nakarai tesque). This was subsequently sliced into 10 μm subsections with a microtome and stained with haematoxylin and eosin (see Figure 5). The prepared slides were observed under magnification using a microscope.

2 - 4 Light microscope measurements

Measurements of medusae in the above sections were carried out at each sampling period. Umbrella width and umbrella height (from the bottom to the top of the umbrella) (Figure 6) of each medusa was measured to the nearest μm. Two measurements were taken for the ampullae, the long diameter and short diameter (Figure 6).

2 - 5 Scanning Electron Microscope observation

Branches bearing ampullae were collected from *M. intricata* colonies on July 28 1997, the soft tissue was removed with 10 % bleach and rinsed in distilled water. The dried preparations were coated with gold using an ion sputter (HITACHI, E 101) and examined with a HITACHI, S-2460 N scanning electron microscope (SEM) at 10 kV.

2 - 6 Observations on released medusae

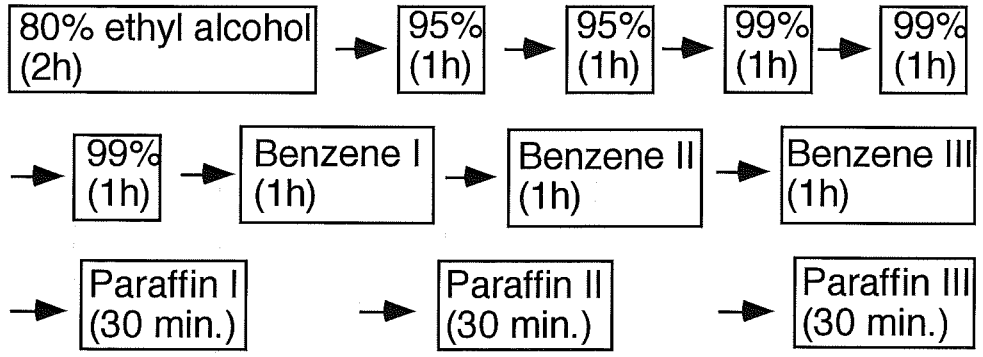
On 20 August 1996, 4 - 5 cm fragments of *M. intricata* bearing ampullae,

1) Sampling

2) Fix (10% formalin sea water)

3) Decalcify (20% acetic acid, 5% formalin, water)

4) Dehydrate



5) Embed

6) Slice (10 μm thickness)

7) Stain

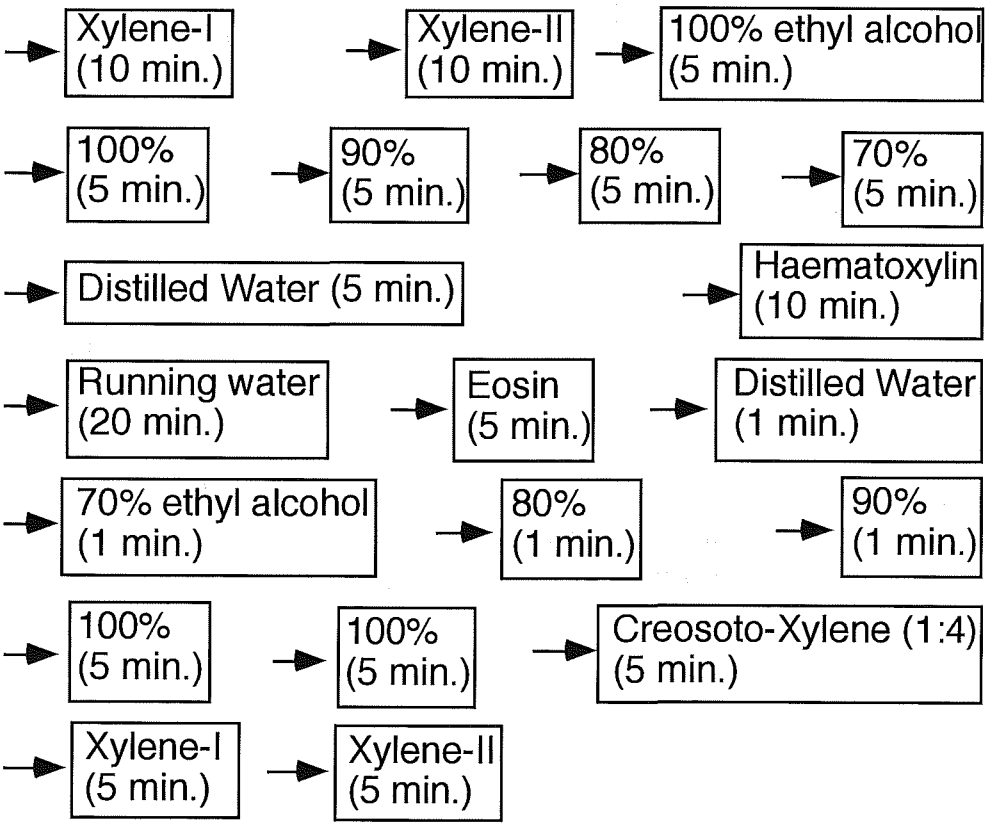


Figure 5. Sequence of the methods used to make histological sections.

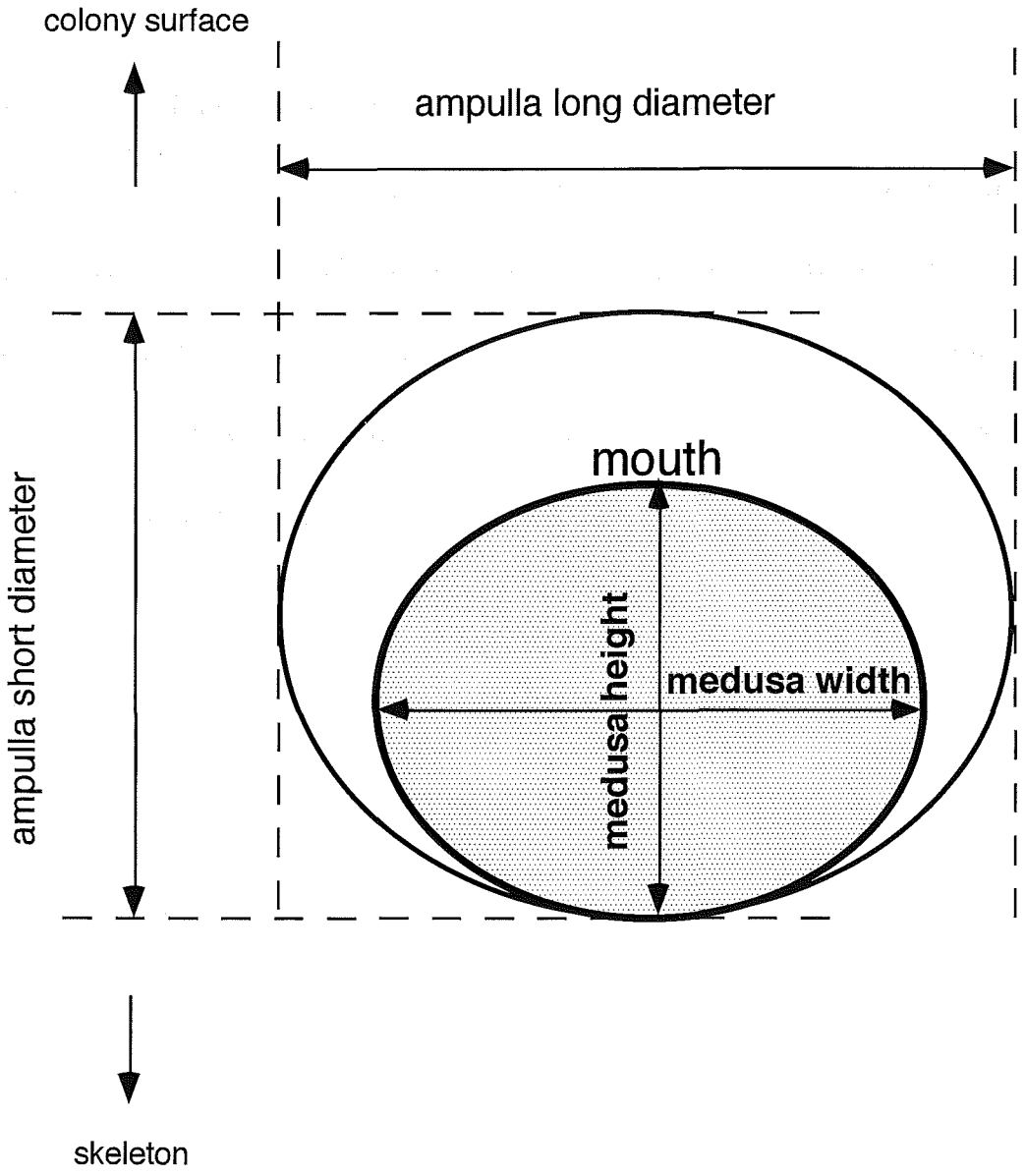


Figure 6. A sketch of the histological section of medusa and ampulla. Measurements were taken along the medusa's umbrella width and height, and the ampulla's long and short diameters.

were collected from the fringing reef of Mizugama on the west coast of Okinawa Island and transported using seawater beakers to the University of the Ryukyus laboratory. Released medusae were anesthetized with 10 % magnesium chloride and fixed in 10 % formalin in seawater. Structures of the specimens were examined with a stereomicroscope. Some of the specimens were embedded in paraffin wax, and sectioned at 5 μm for histological examination.