琉球大学学術リポジトリ

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

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3. RESULTS

3 - 1 Development of medusae and ampullae

Ampullae and medusae take almost elliptical forms, therefore their development was measured as change in elliptical area (long diameter x short diameter x 0.7854, see Figure 6). Changes in the size of *M. intricata* and *M. tenella* medusae are shown in Figures 7 and 8. To compare the growth rate between species, the regression slopes were compared using the natural logarithm of the mean medusa area for the samples taken in the same period (from May 9 to May 27) (Figure 9). Medusae from both species consistently increased in size over time (*M. intricata* R^2=0.897, N=254, $p<0.0001$, *M. tenella* R^2=0.911, N=129, $p<0.0001$, Figure 9), and there was no significant difference between the two regression slopes (comparison of two regression slopes, $p>0.05$), although *M. intricata* had larger medusae than *M. tenella* (comparison of two regression intercepts, $p<0.05$).

Medusae size was compared between the top and the base of *M. intricata* colonies on each date (May 9, May 16, May 22, May 27). Samples from three colonies were used because sample numbers of two colonies were too small for comparative purposes. There was no significant difference in the

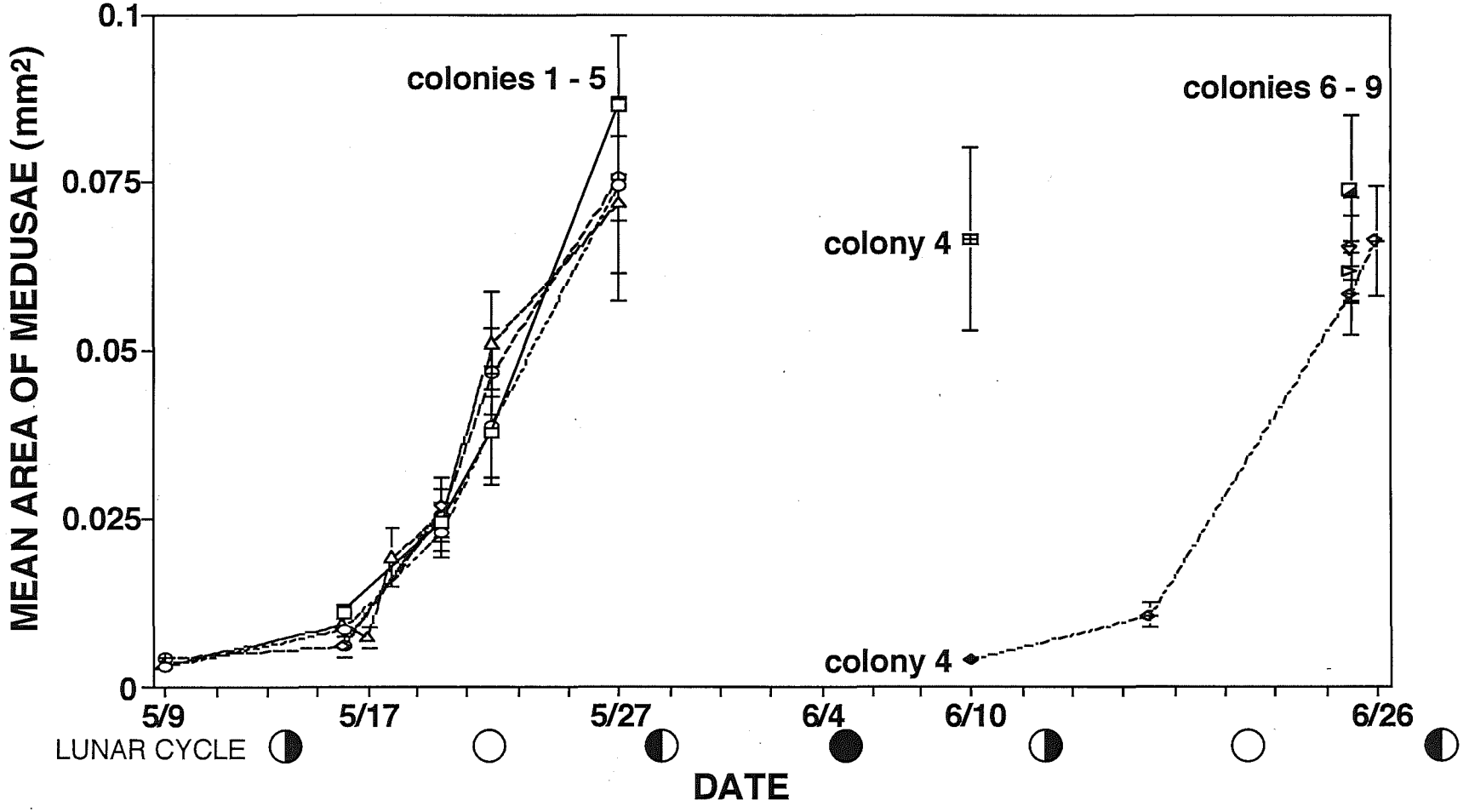


Figure 7. Change in size of medusae in *M. intricata* colonies from 9 May to 26 June 1997. Symbols indicate different colonies measured, and ◯ indicates full moon.

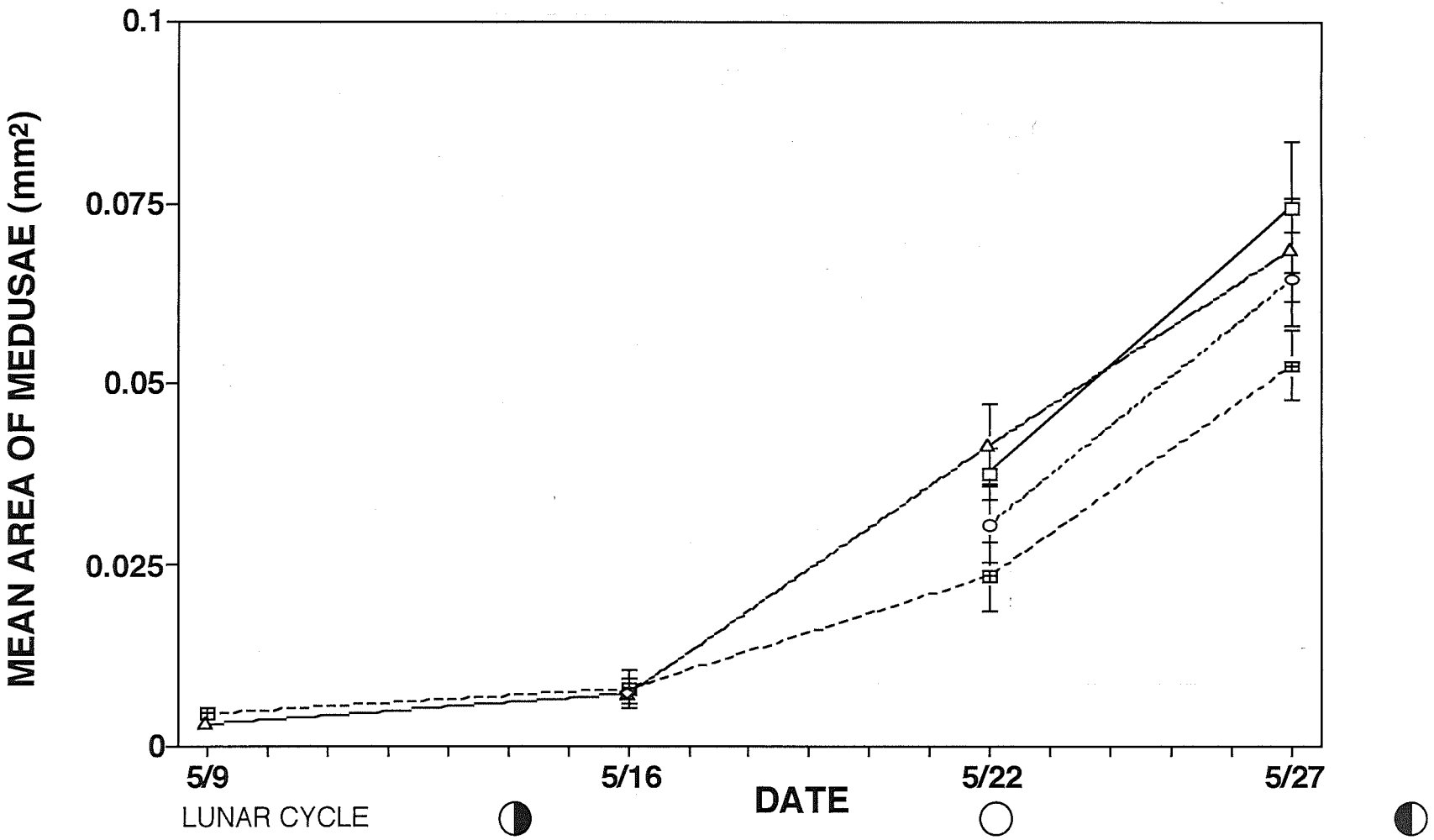


Figure 8. Change in size of medusae in *M. tenella* colonies from 9 to 27 May 1997. Symbols indicate data from different colonies, and ◯ indicates full moon.

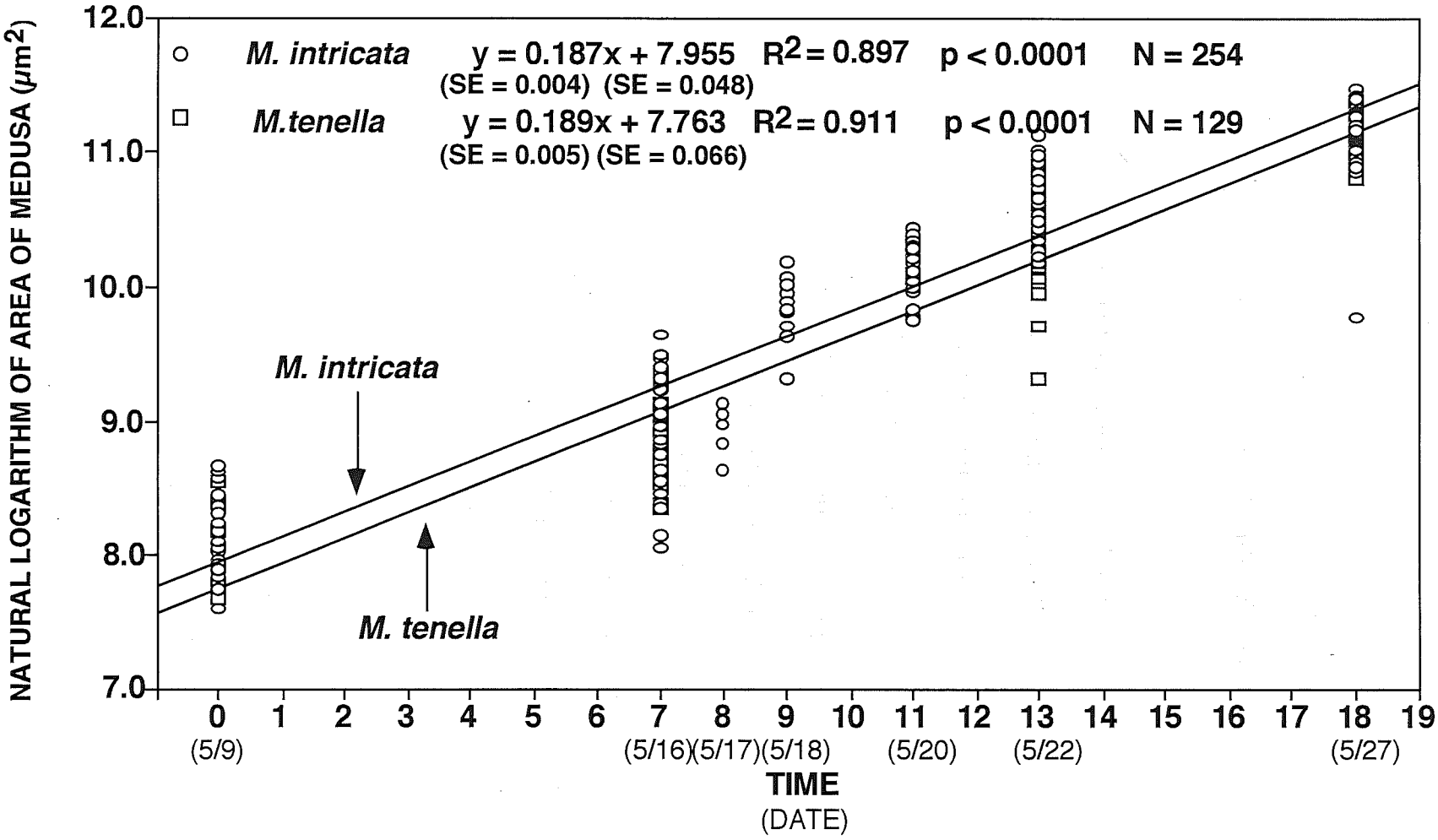


Figure 9. *M. intricata* and *M. tenella*. Correlation between time and medusa area. Medusa area was transformed into natural logarithms because the relationship was exponential. There was no significant difference between the regression slopes ($p > 0.05$).

size of medusae located either in the top or at the base of the colonies on 9 and 16 May (Mann-Whitney's U test, $p>0.05$). However, medusae size at the base was significantly larger than the top, in one colony on May 22, and in two colonies on May 27 (Mann-Whitney's U test, $p<0.05$).

The developing medusae was synchronized within and among colonies for both species. The first recognizable appearance of the earliest forms of medusae development were detected both for *M. intricata* and *M. tenella* on May 9, 1997 in the histological sections, classified here as stage I (Figure 10). At this stage, there were no clear ampullae. Distinct ampullae and early medusa were found on May 16 (7 days after first appearance) classified here as stage II (Figure 11). On May 22 (13 days later), they had developed into easily recognizable medusae (stage III), which had either male or female gonads (Figure 12). Subsequent studies showed that in the marked colonies of *M. intricata*, there were 4 males and 1 female. In contrast, all the *M. tenella* colonies were female. On May 27 (18 days later), medusae had developed into their full apparent size (stage IV), filling the ampullae almost entirely (Figure 13). Many zooxanthellae were found inside the medusae. It was observed that the mouths of all the medusae were situated toward the surface of each colony. In those specimens, there were some open ampullae (Figure 13c).

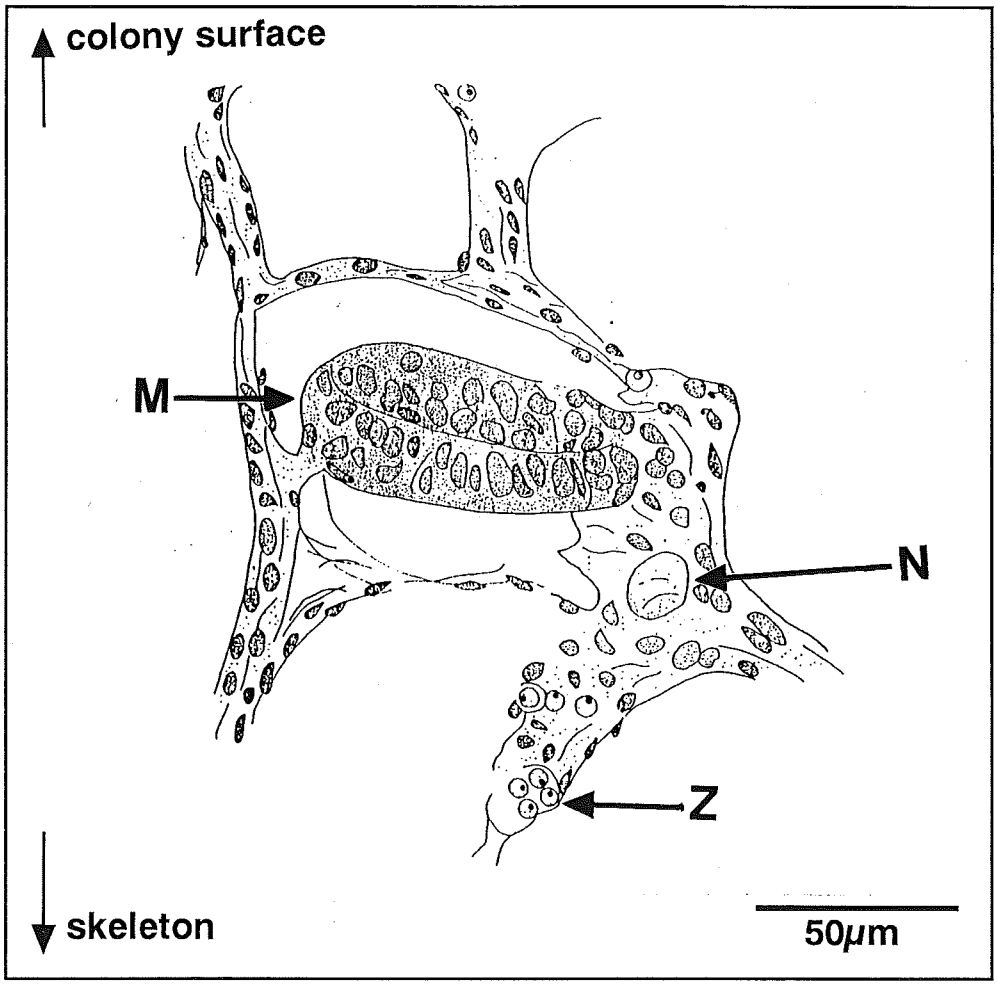


Figure 10. Transverse section across *M. intricata*. The earliest stage of development (stage I) on 9 May 1997. An ampulla is not clear at this stage. M: medusa, N: nematocyst, Z: zoothanthella

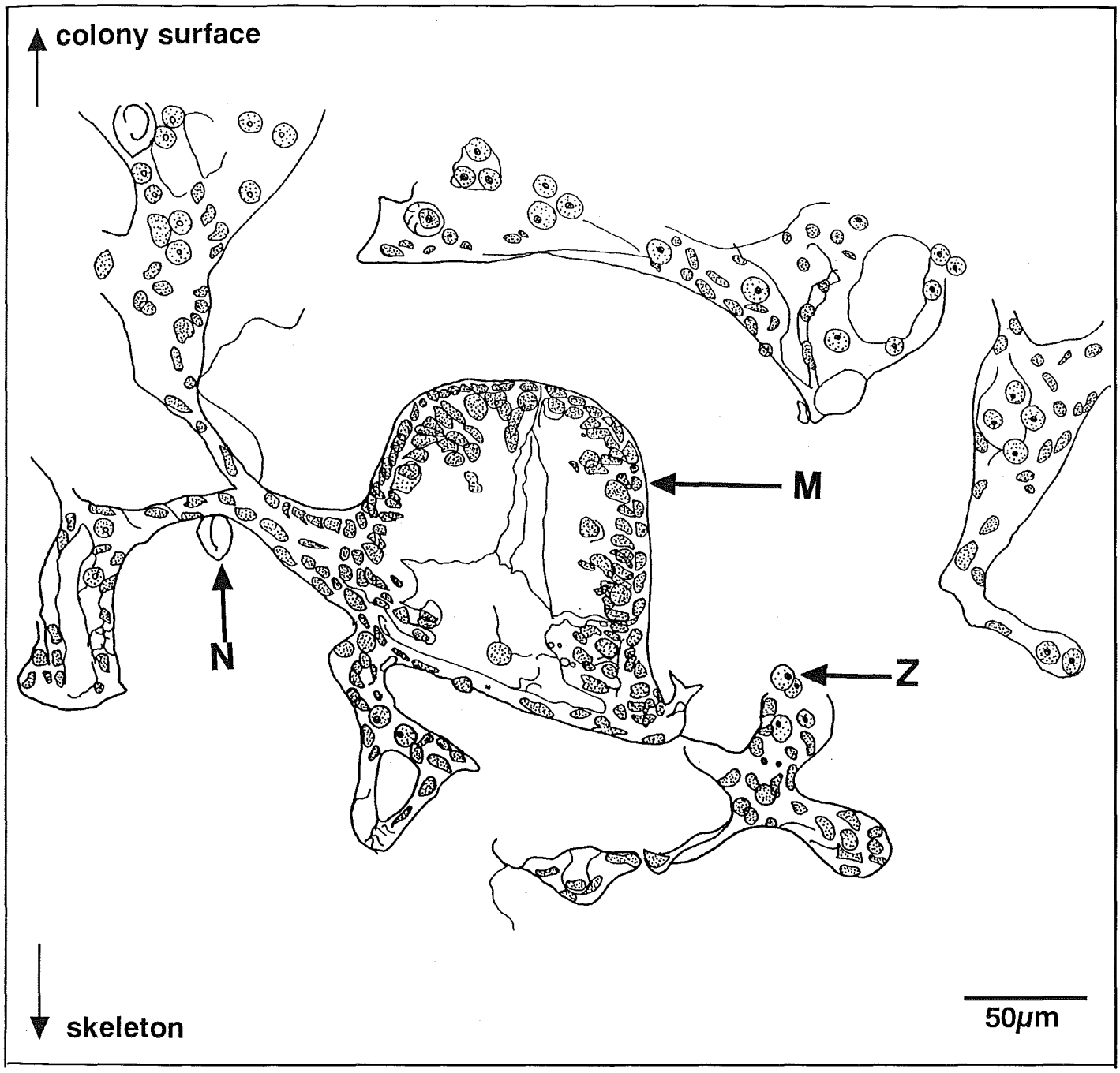


Figure 11. Transverse section across *M. intricata*. An early stage of medusa development (stage II) on 16 May 1997. A distinct ampulla is formed around the medusa. M: meudsa, N: nematocyst, Z: zooxanthella.

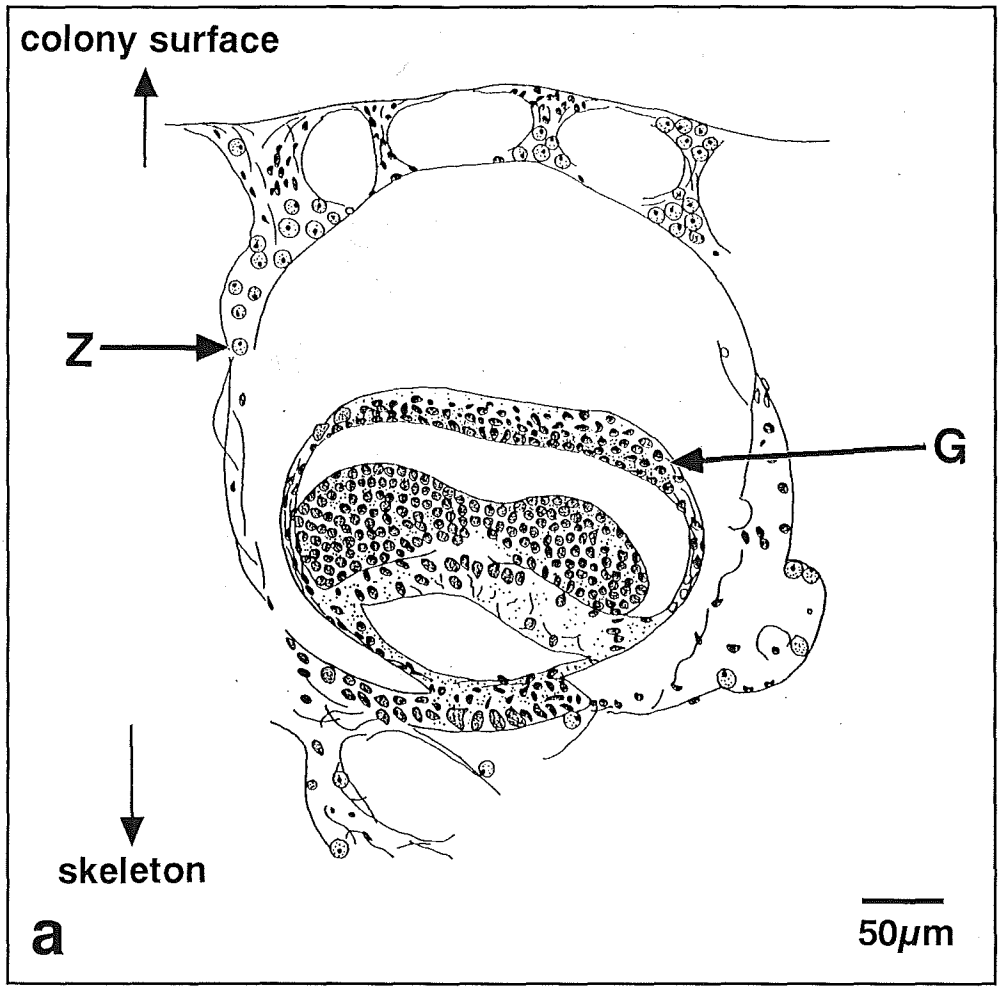
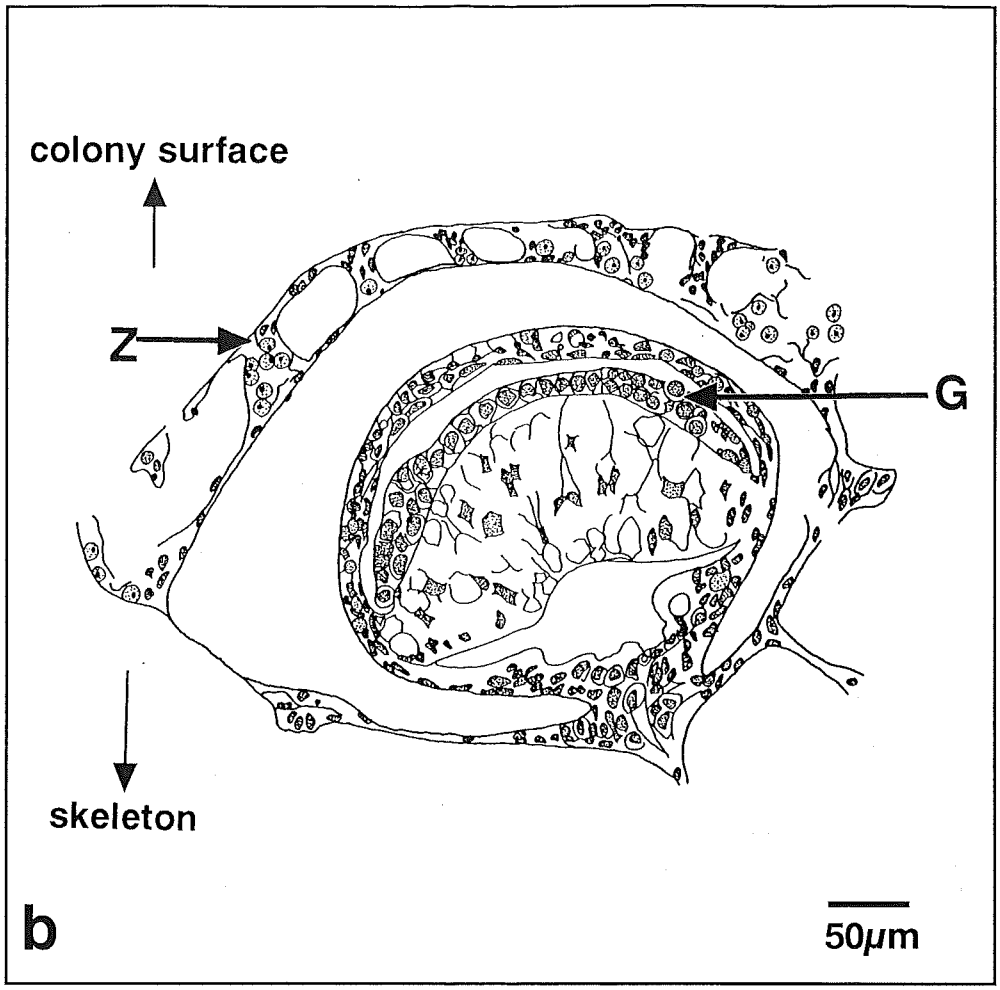
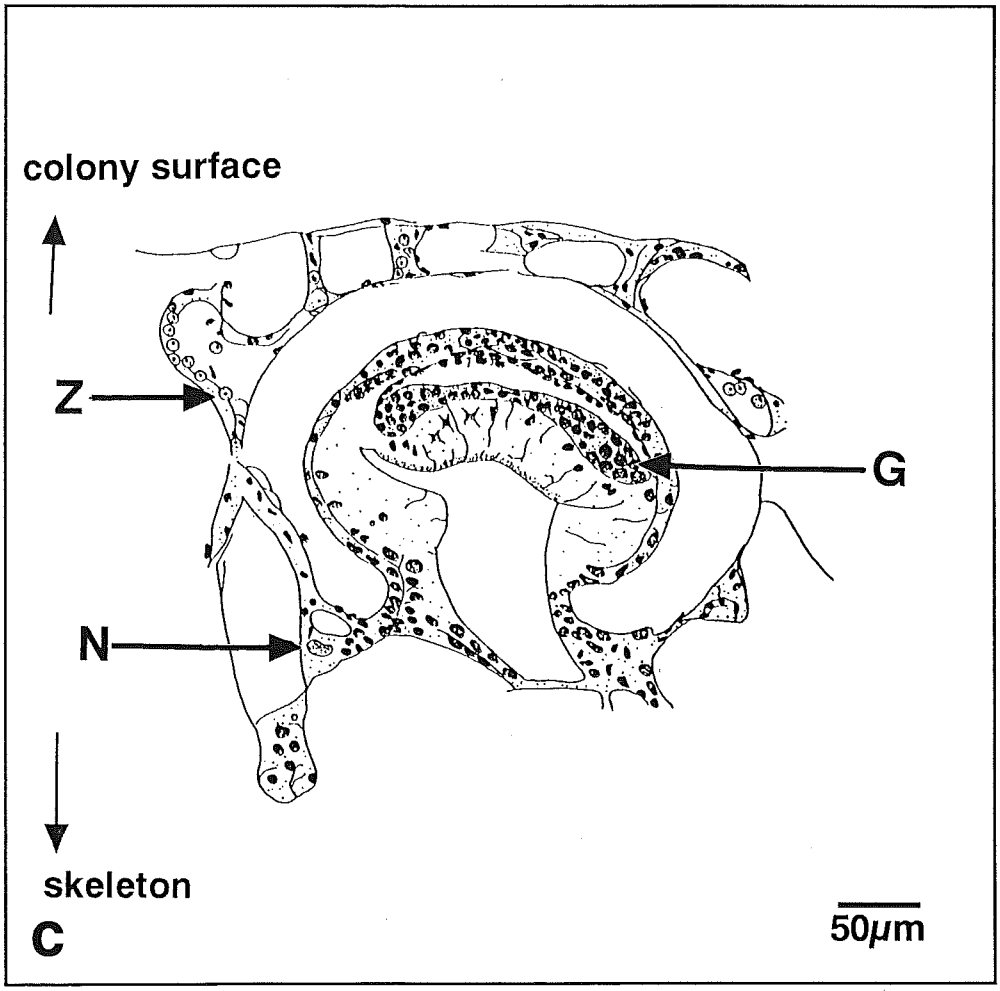


Figure 12. Stage III on 22 May 1997. **a)** A male medusa of *M. intricata*. **b)** A female medusa of *M. intricata*. **c)** A female medusa of *M. tenella*. G: gonad, Z: zooxanthella.


colony surface
Z
G
skeleton
b
50µm


colony surface
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C
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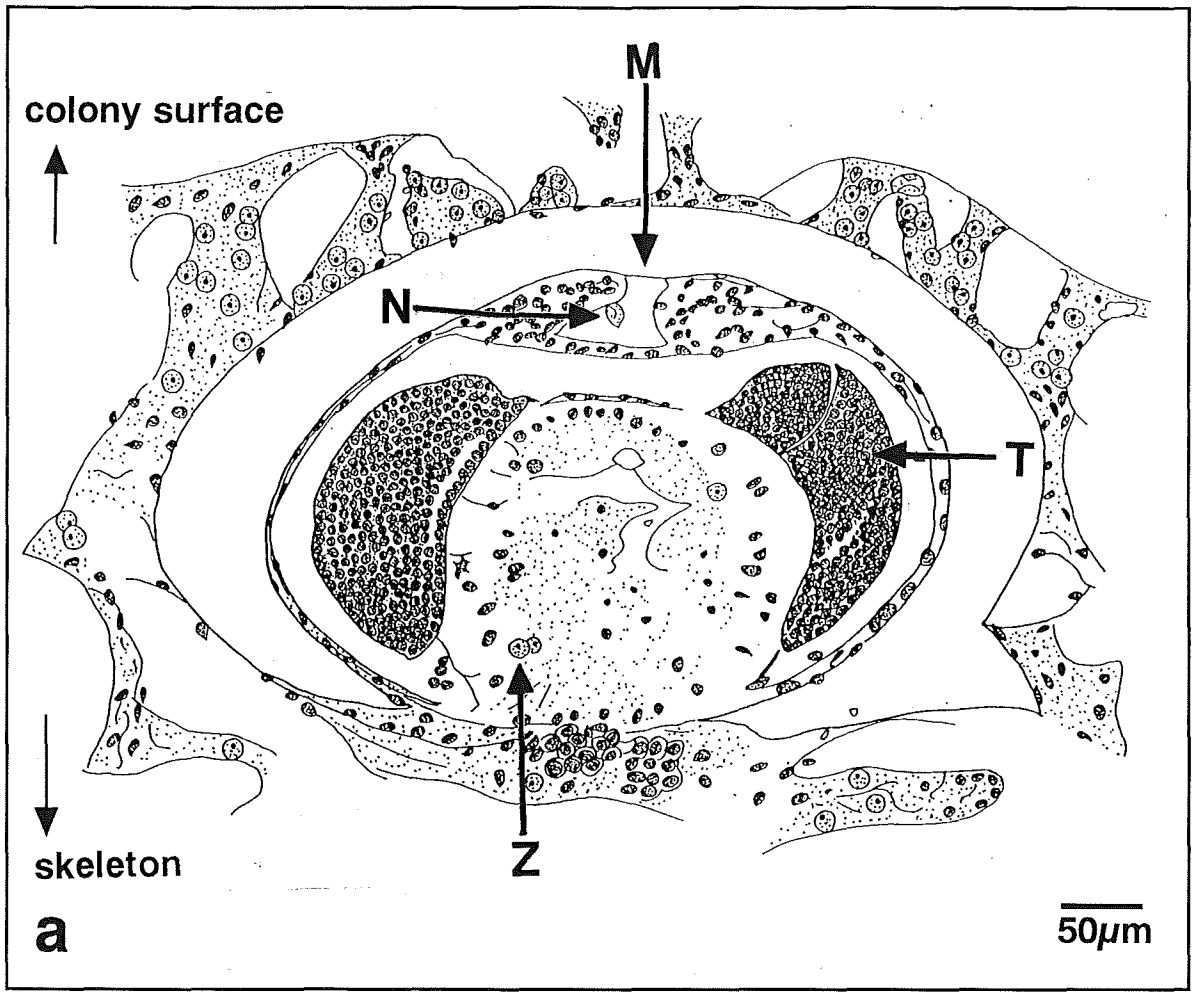
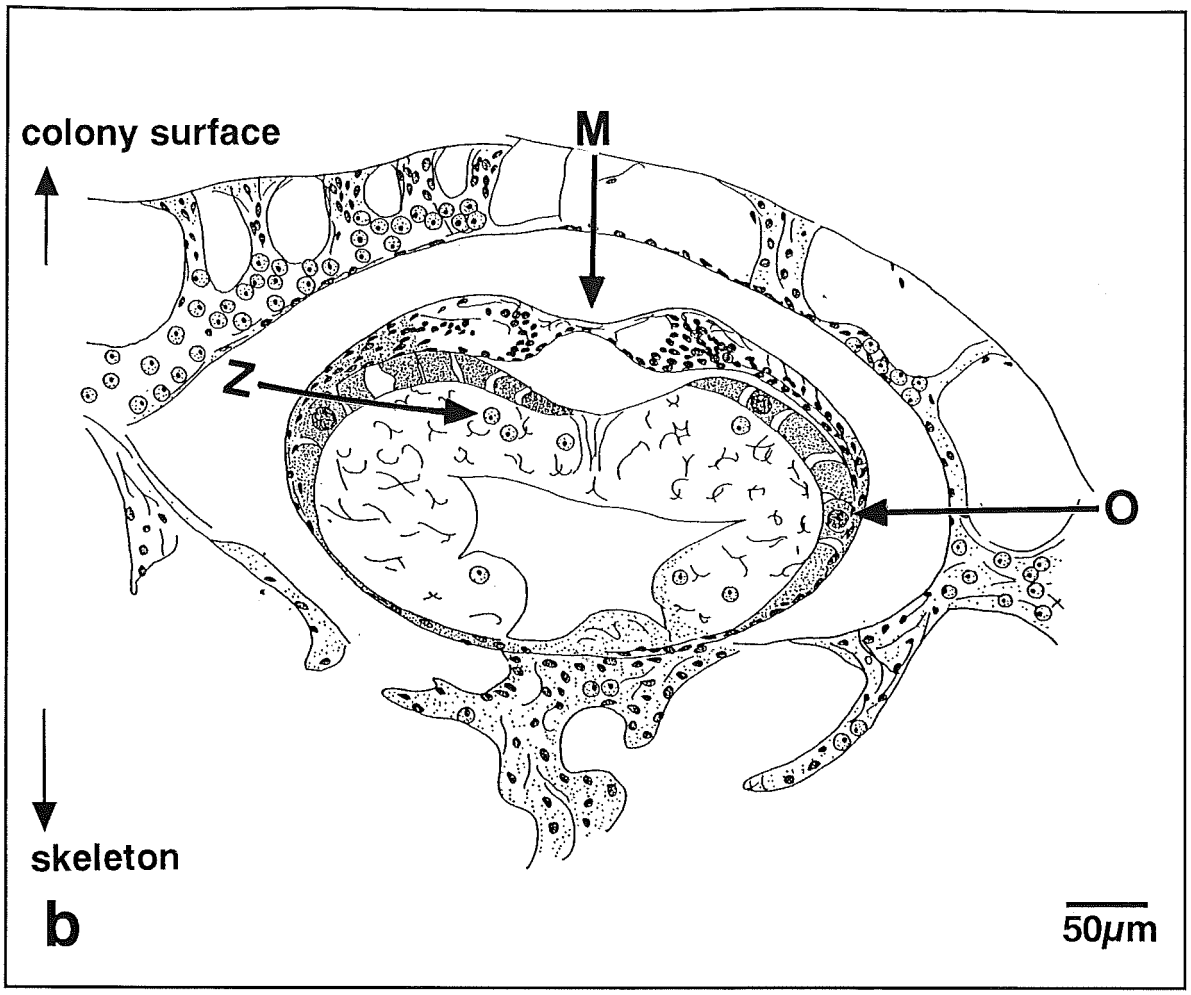
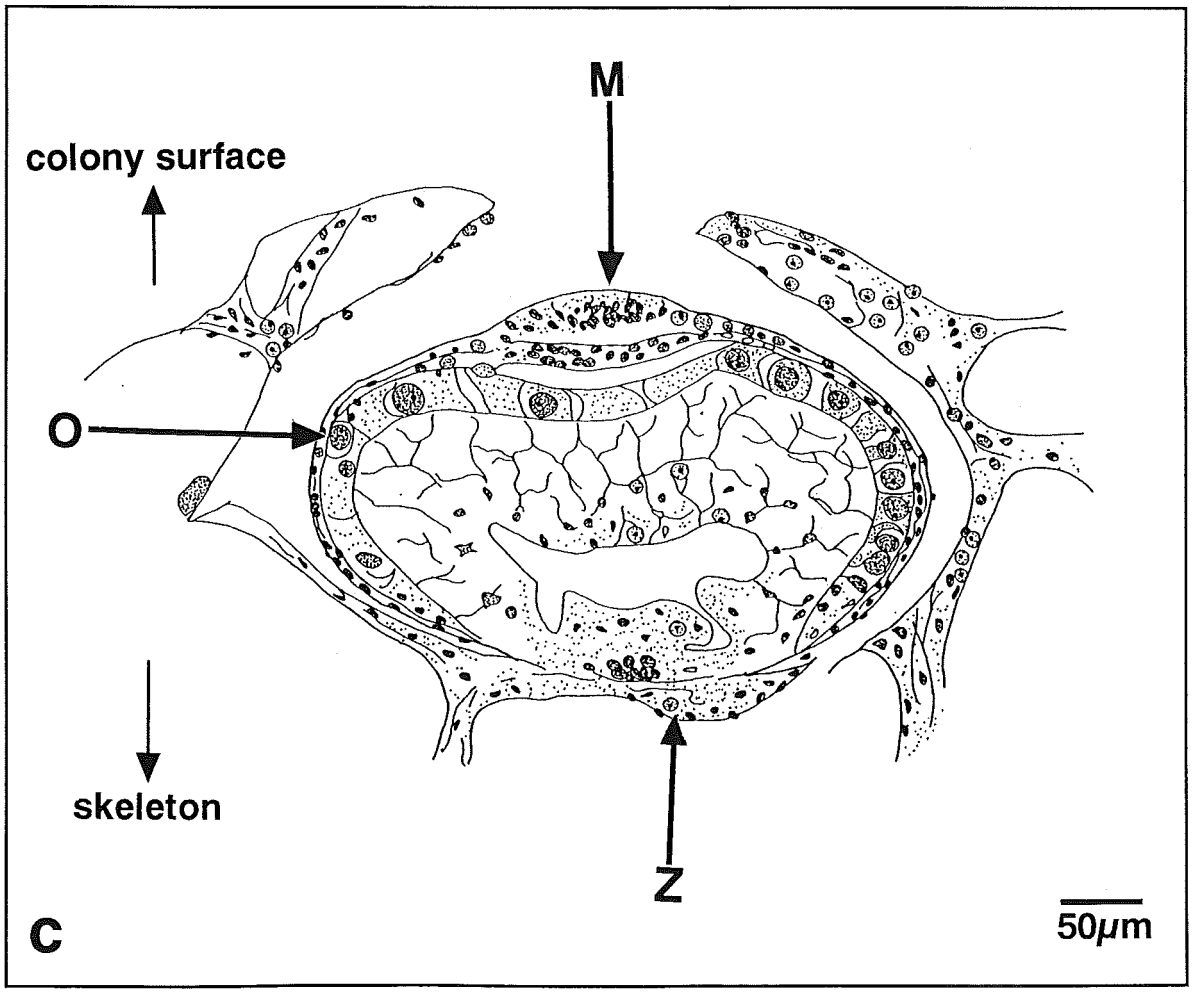


Figure 13. Stage IV on 27 May 1997. **a)** A male medusa of *M. intricata*. **b)** A female medusa of *M. intricata*. **c)** A female medusa of *M. tenella*. M: mouth of umbrella, T: testis, O: oocyte, N: nematocyst, Z: zooxanthella.


colony surface
M
Z
O
skeleton
b
50µm


M
colony surface
O
skeleton
Z
c
50µm

On June 4 (26 days later), no medusae and empty ampullae were observed in most histological sections of all the marked colonies. Compared to swollen ampullae on May 27, field observations on June 4 showed that the ampullae had been broken and become flat. Figures 14 and 15 show photos of histological sections of medusae in each developmental stage from 9 to 27 May.

New ampullae seemed to appear on June 10 on the surface of *M. intricata* "colony 4". It was difficult to distinguish new and old ampullae with the naked eye because they were mixed and aggregated. There were both immature (stage I) and mature medusae (stage IV) (Figure 7) on June 10. Only early medusae were found (stage II) on June 17 (7 days later). On June 26 (16 days later), there were mature female medusae present (stage IV). The other four (marked) colonies of *M. intricata,* and all the marked colonies of *M. tenella,* had no apparent ampullae after May 27. However, nearby *M. intricata* colonies had numerous ampullae on the surface of the colonies. Therefore, four extra colonies were sampled, near the marked colonies on June 26. No medusae were present in sections after July 1, 1997, until October when the present study ended. Ampullae left on the colony surface gradually faded away completely in one month.

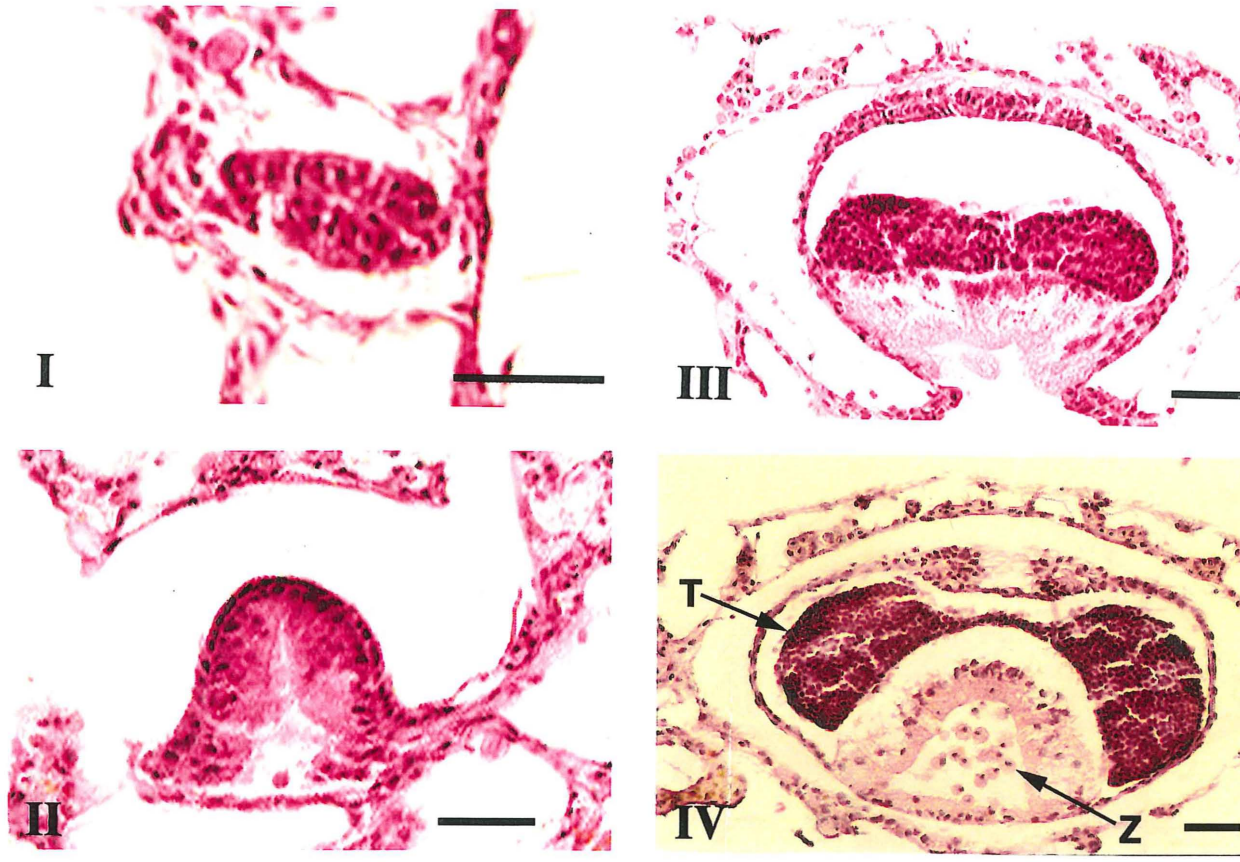


Figure 14. *Millepora intricata.* The developmental stages of male medusa. All scale bars are 50 μm. I ~ IV indicate each developmental stage. T: testis, Z: zooxanthella.

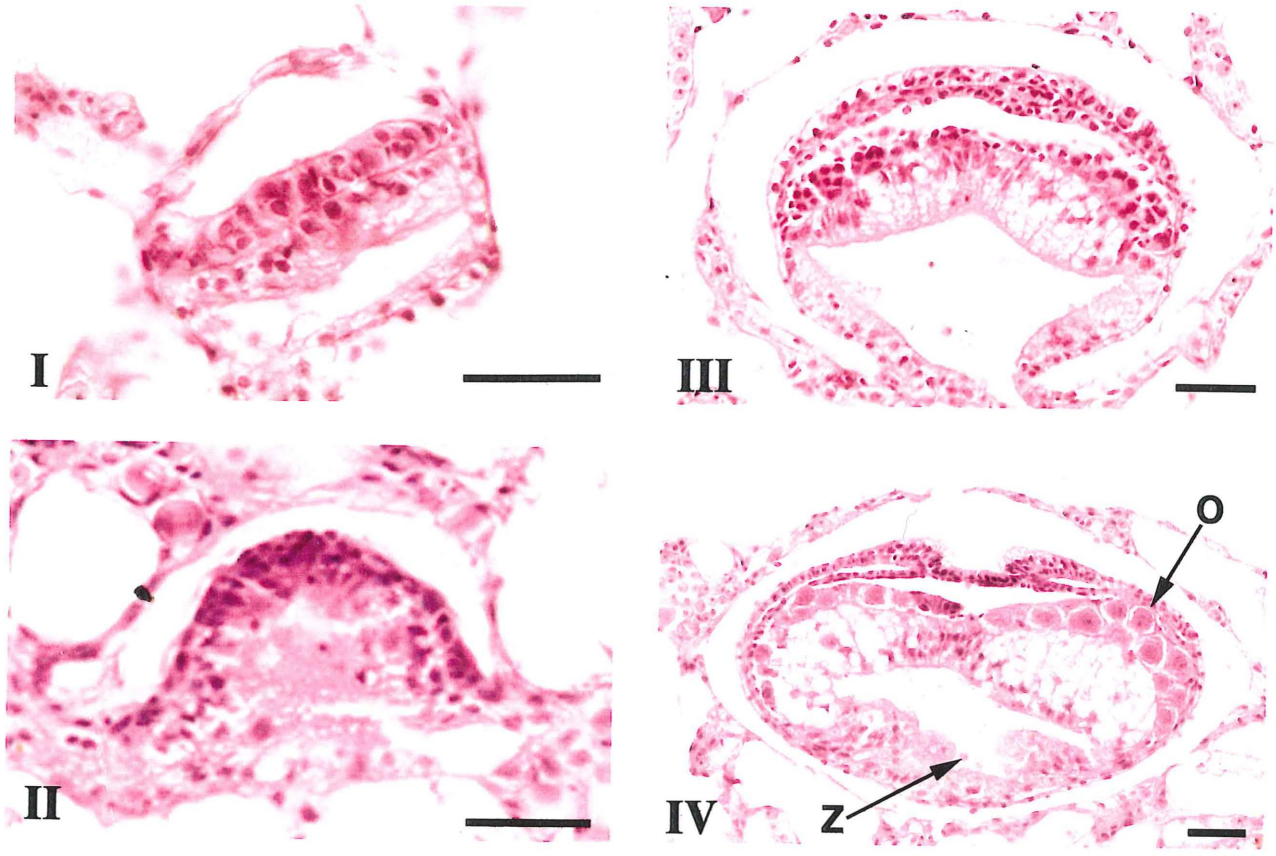


Figure 15. *Millepora tenella*. The developmental stages of female medusa. All scale bars are 50 μm. I ~ IV indicate each developmental stage. O: oocyte, Z: zooxanthella.

There was a strong correlation between medusa size and ampulla size in both species (*M. intricata* R^2=0.93, N=366, p<0.0001, *M. tenella* R^2=0.93, N=130, p<0.0001) (Figures 16 and 17). Therefore, it is suggested that ampullae increase in size as medusae grow.

3 - 2 Synchronization of medusae development

To examine synchronization among colonies of change in medusa size, samples of *M. intricata*, taken from 9 to 27 May 1997, were tested. Data from "colony 1" and "colony 2" were excluded because the data sets were unbalanced. There were no significant differences between "colony 3", "colony 4", and "colony 5" (Two-way Repeated Measures ANOVA, interaction effect p=0.331, Figure 19). Therefore, this preliminary data suggests that change in medusa size was synchronized among these three colonies of *M. intricata.* However, change in medusa size was not synchronized for two colonies of *M. tenella* (Two-way Repeated Measures ANOVA, interaction effect p=0.0002, Figure 20).

3 - 3 Structure of ampullae

Ampullae were visible as small, white, blisters on the surface of the marked *M. intricata* colonies for the first time on 16 May 1997 (Figure 21a). Some of the marked colonies had a large number of ampullae (at

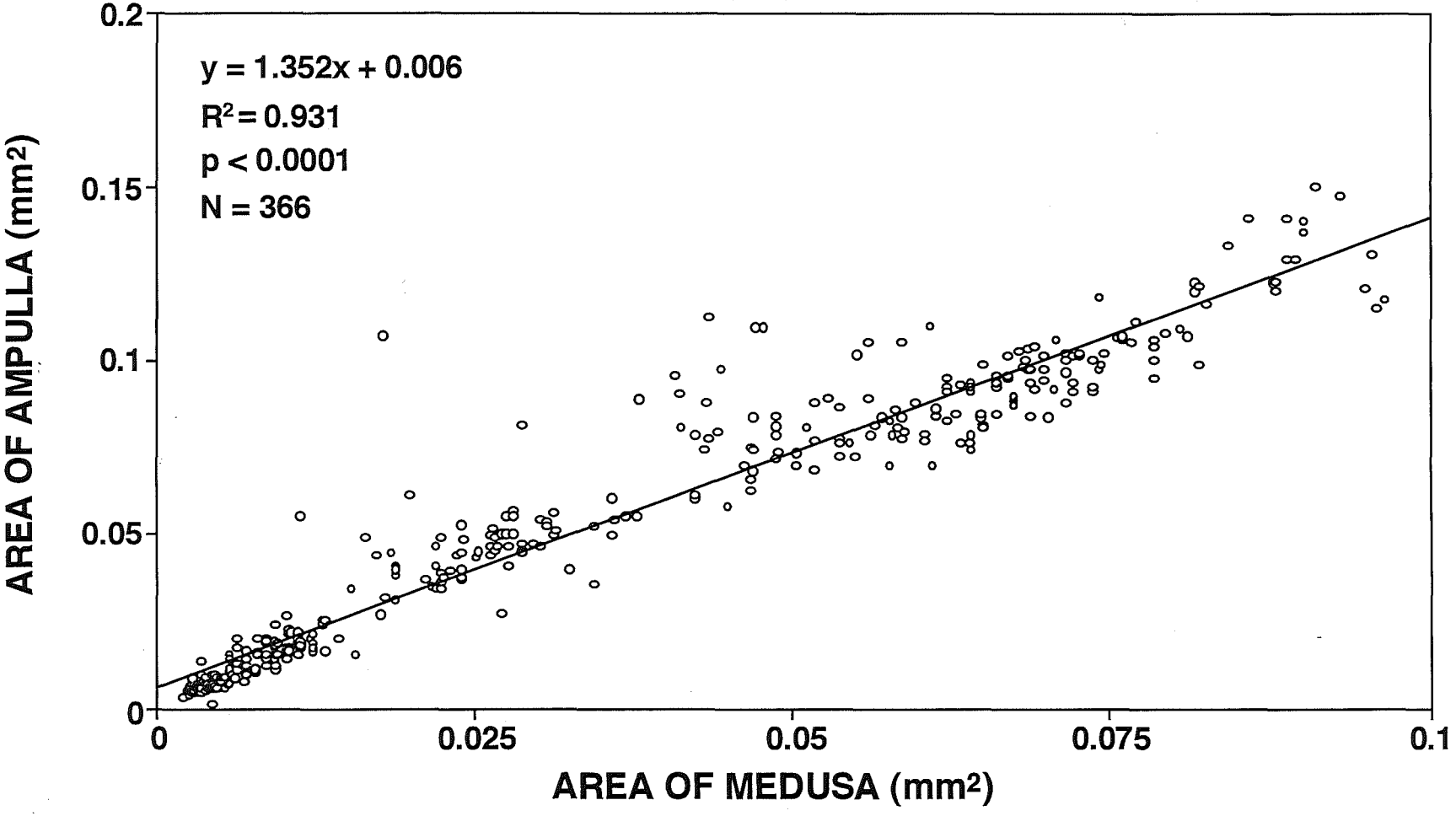


Figure 16. *Millepora intricata.* Correlation between medusa and ampulla size.

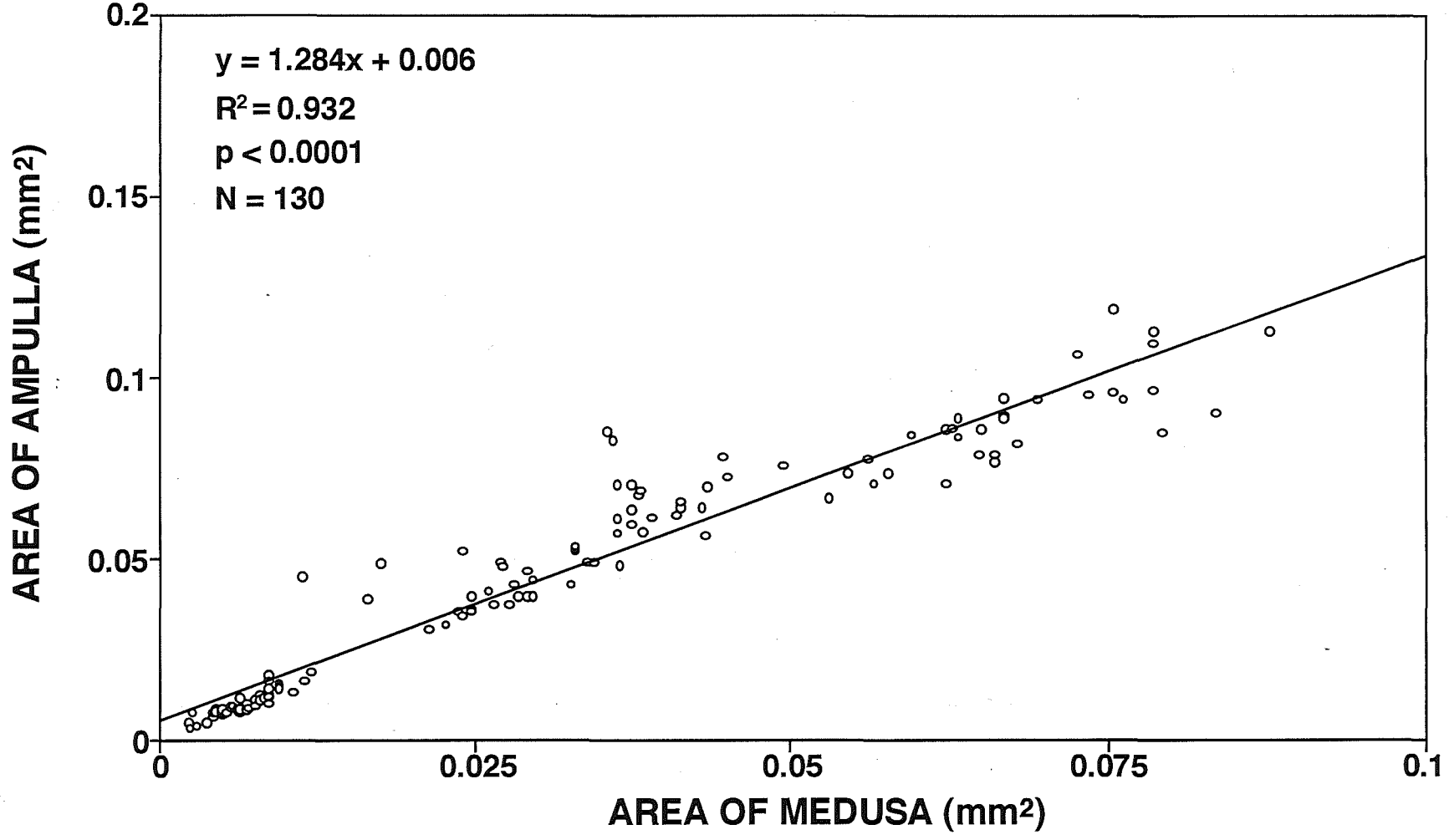


Figure 17. *Millepora tenella*. Correlation between medusa and ampulla size.

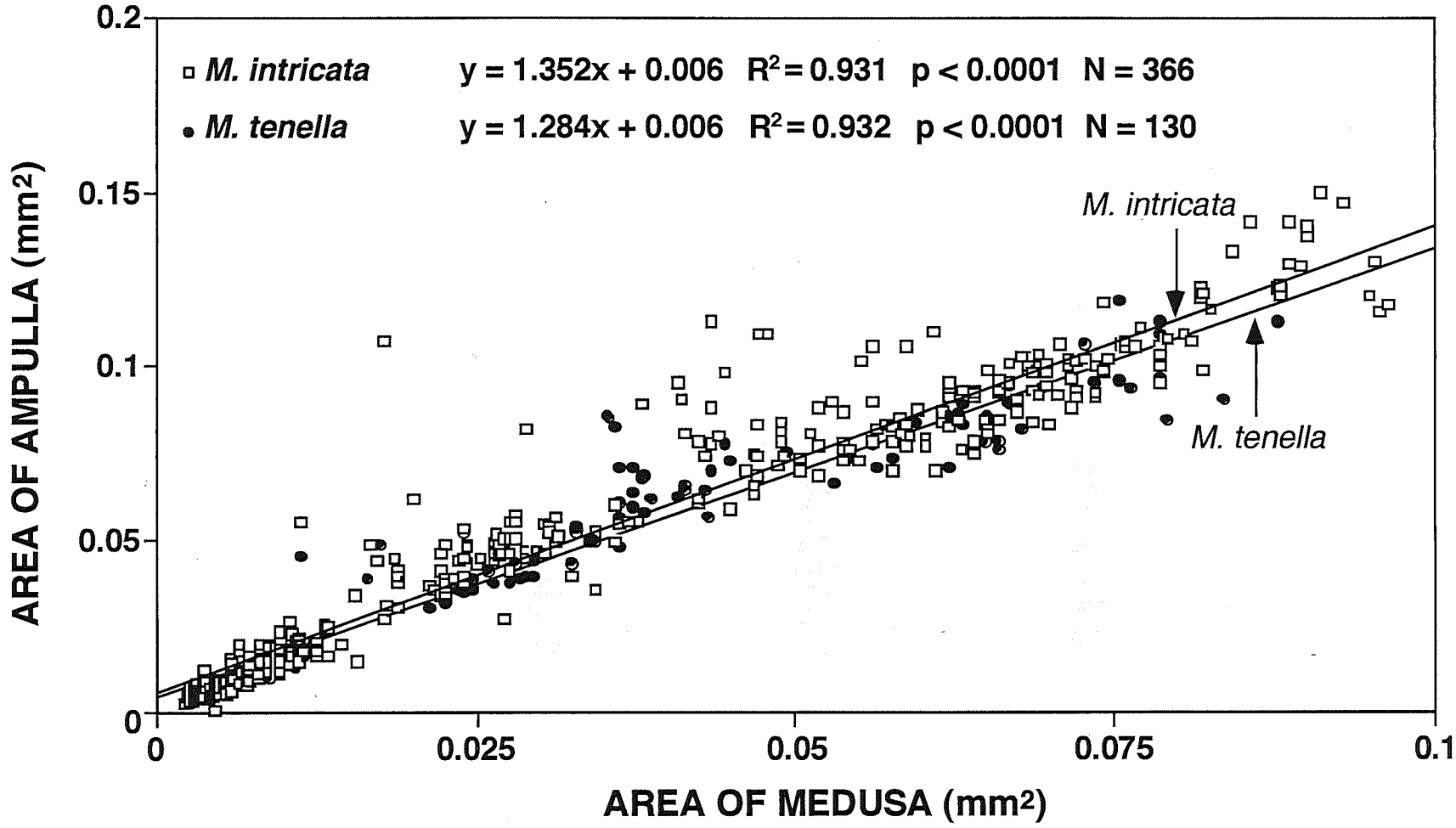


Figure 18. Correlation between medusa and ampulla size of *M. intricata* and *M. tenella*.

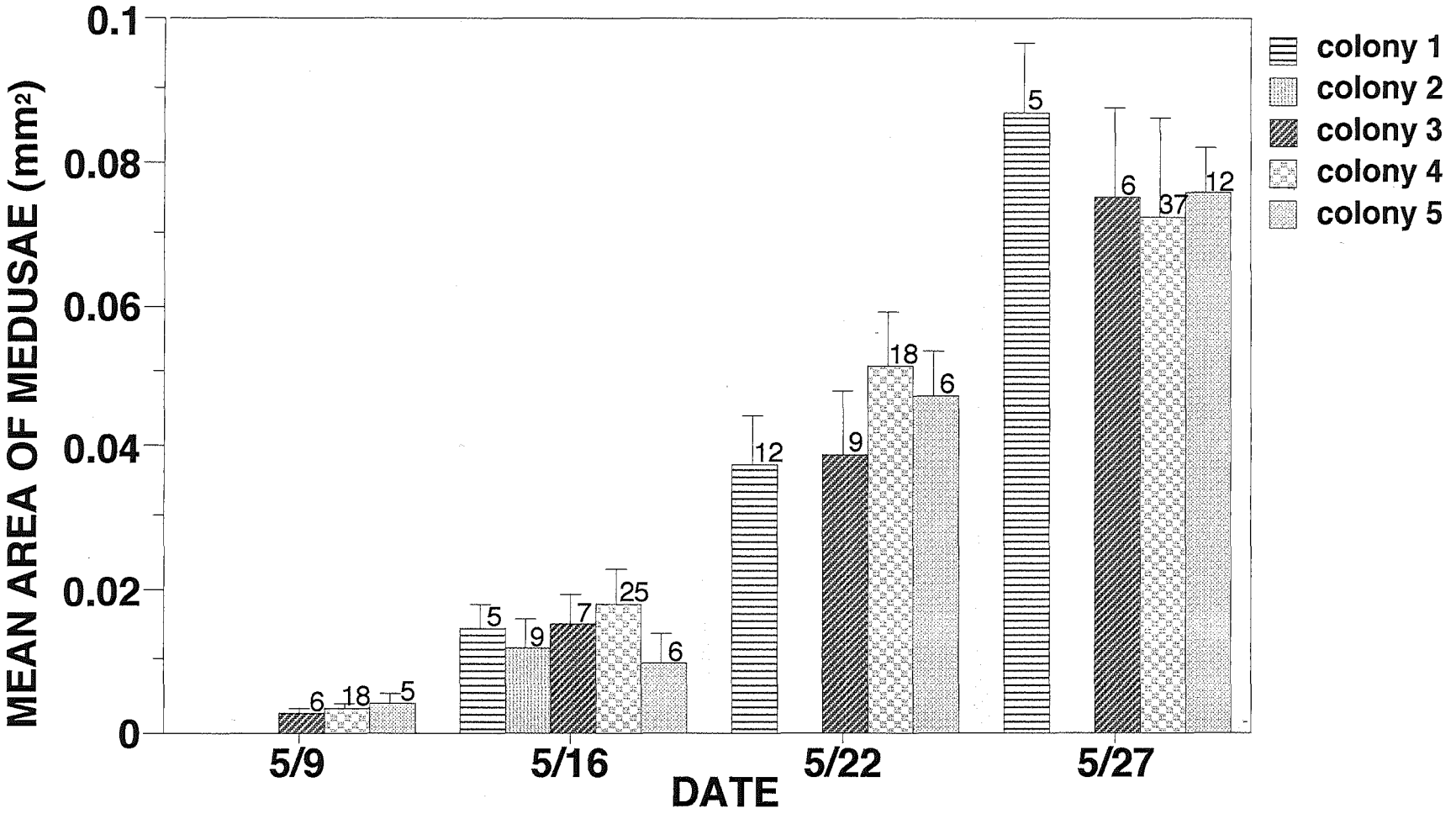


Figure 19. *Millepora intricata*. Mean area of medusae of colonies 1 - 5 on each date. Sample number is different among the colonies. There were no significant differences between "colony 3", "colony 4", and "colony 5" according to time (Two-way Repeated Measures ANOVA, interaction effect $p = 0.331$). Numbers above the bars are the sample numbers.

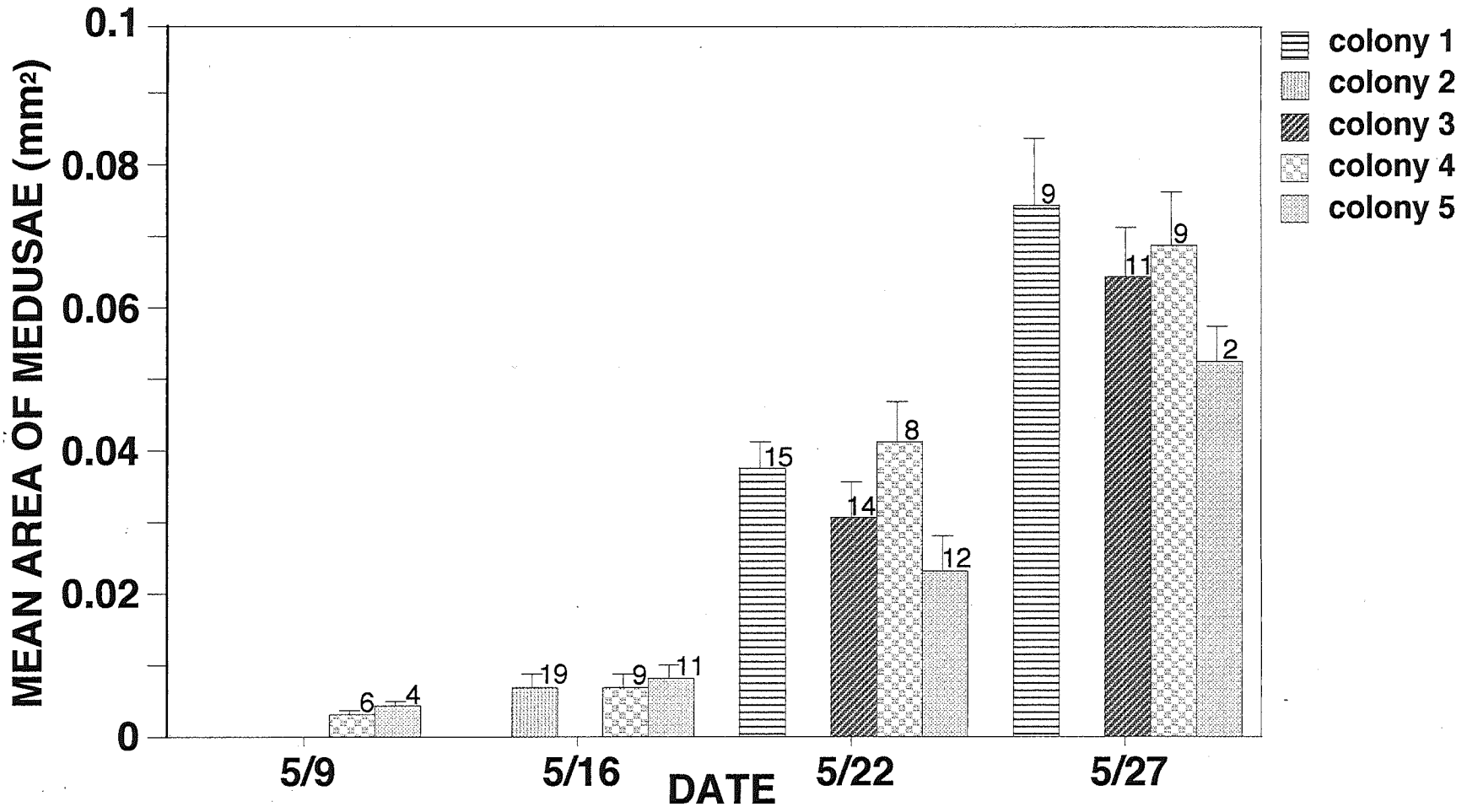


Figure 20. *Millepora tenella.* Mean area of medusae of colonies 1 - 5 on each date. Sample number is different among the colonies. Test was valid only for "colony 4" and "colony 5", and there was a significant difference between these colonies according to time (Two-way Repeated Measures ANOVA, interaction effect $p = 0.0002$). Numbers above the bars are the sample numbers.

most 15 ampullae per cm^2), most had a smaller number. There were no ampullae between 0 - 1.5 cm from the tip of each branch (Figure 21b), nor on the inward facing branches where pigment coloration was faint. The ampullae of *M. intricata* colonies appeared to become clear, and more convex, during the course of medusae development. The number of ampullae varied considerably among colonies.

On the other hand, ampullae of *M. tenella* colonies started to appear on May 27, but were less clear than *M. intricata* ampullae. The size of *M. tenella* ampullae, on May 27, was significantly smaller than the size of *M. intricata* ampullae (Mann-Whiteny's U test, $p<0.0001$). There was also no ampullae at the growing tip, however, ampullae were evident on both branch sides and the encrusting base of each colony.

It was apparent that ampullae develop as distinct pores (Figure 22). The skeleton covering of the ampullae was arranged in a radial manner (Figure 23a), with the center pierced by a small opening. The size of *M. intricata* ampullae was approximately 0.5 mm. As an ampulla develops, it barely rises above the general surface (Figure 23b) and the central hole becomes larger (Figure 24). Figure 24d shows the remnants of an ampulla after the medusa release.

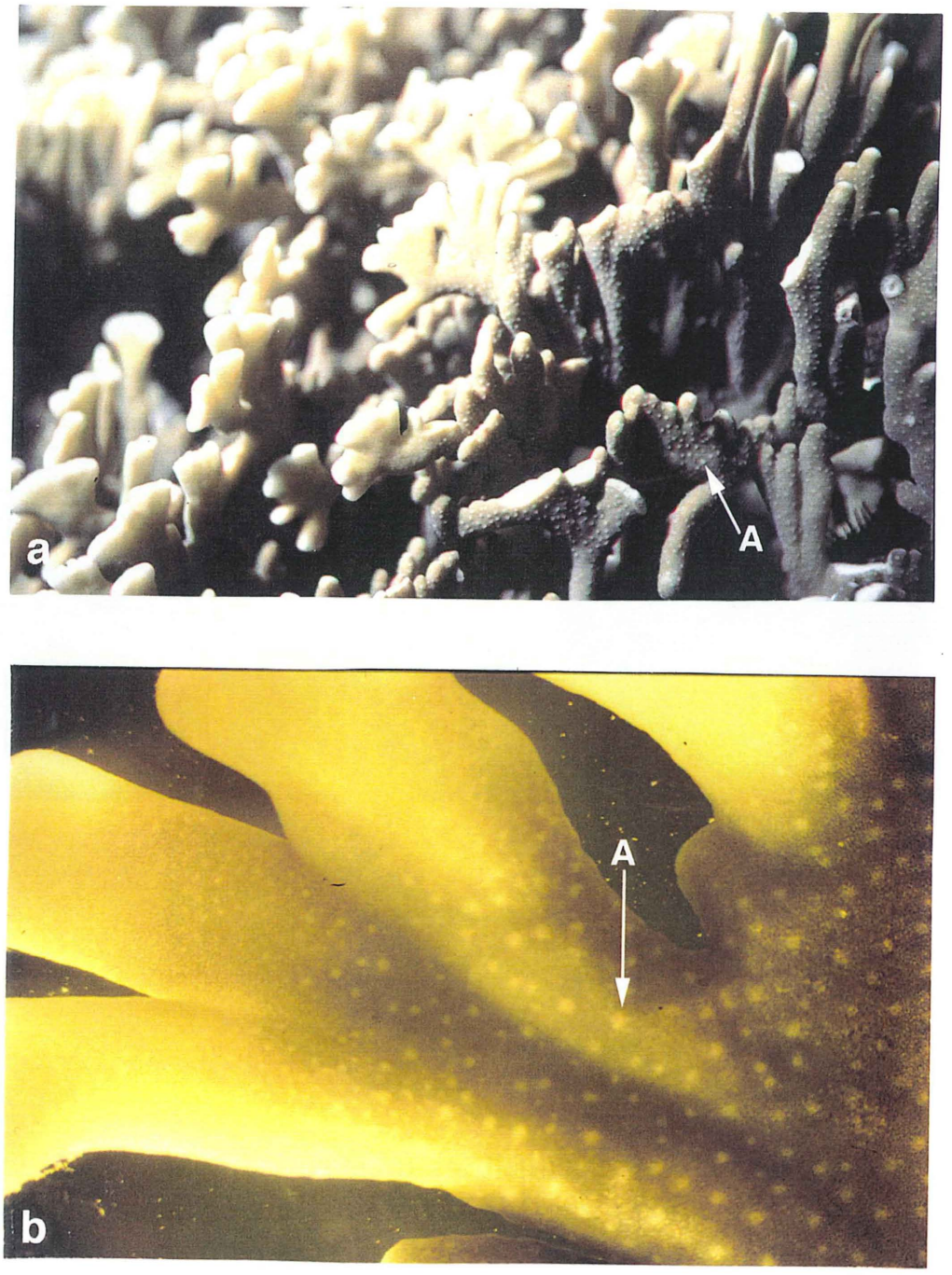


Figure 21. Ampullae on the surface of *M. intricata*: **a)** Ampullae are visible as small white blisters; **b)** Ampullae present, but not apparent between 0 - 1.5 cm from the tip of each branch. A: ampulla.

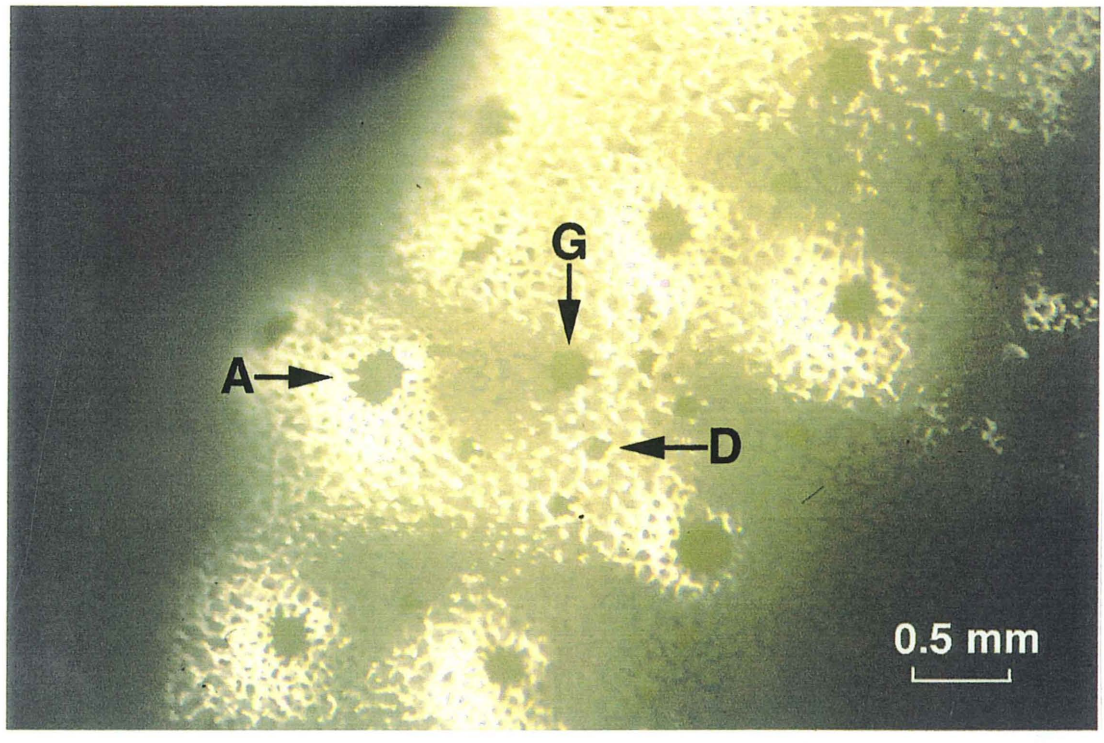


Figure 22. Ampullae develop as distinct pores; when medusae are released the ampullae are larger than the gastropores and dactylopores; A: ampulla, G: gastropore, D: dactylopore.

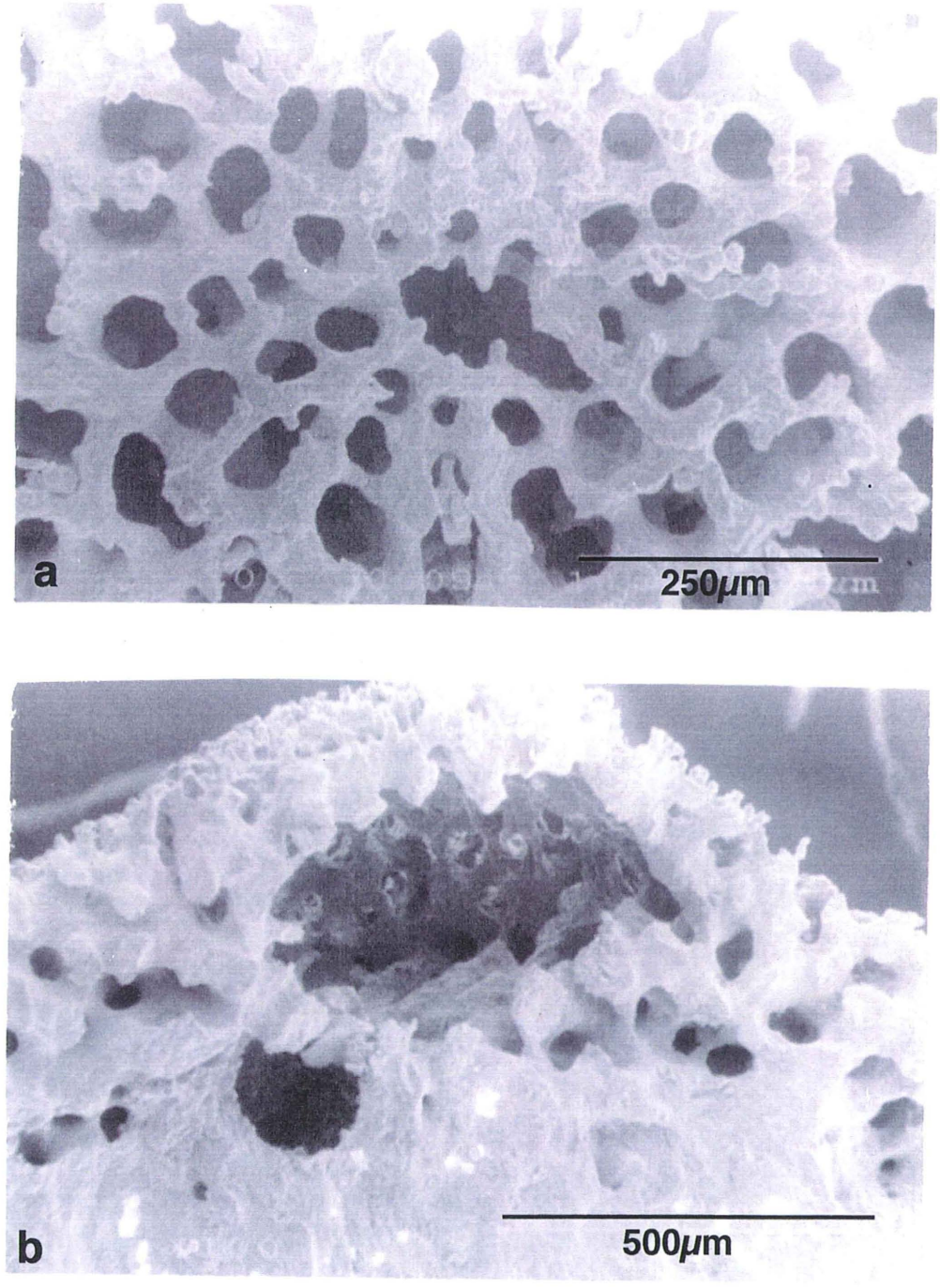


Figure 23. SEM (Scanning Electron Microscope) photographs of ampullae. a) The skeletal covering of ampulla consists of a mass of trabeculae which are arranged in a radial manner; b) Vertical section of developed ampulla.

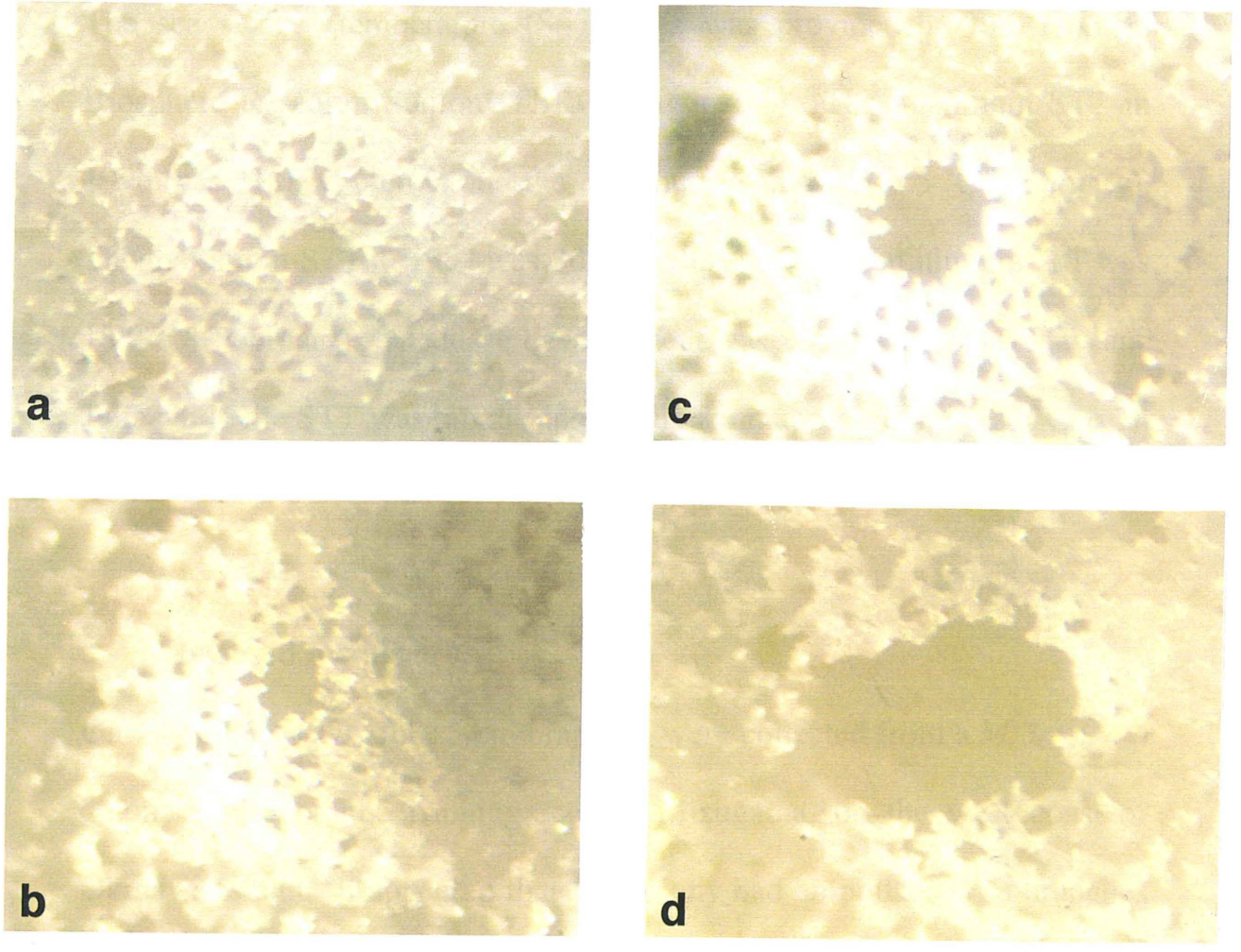


Figure 24. Various stages in the development of ampullae of *M. intricata*. **a**) and **b**) Early-stage ampulla with loose covering of radiating trabeculae and central pore; **c**) Late-stage ampulla with a larger central pore; **d**) Empty ampulla after release of medusa.

3 - 4 Water temperature

The sea surface temperature (SST) data was provided by the Tropical Biosphere Research Station (TBRC) (Figure 25). The maximum STT in 1996 was 31.0 ˚C (June), and the minimum STT was 18.5 ˚C (February). The maximum STT in 1997 was 30.6 ˚C (July), and the minimum STT was 18.9 ˚C (February). Medusae development was observed during May and June while the STT was rapidly increasing from approximately 26 ˚C to 28 ˚C.

3 - 5 Released medusae

On 19 August 1996, four 3 cm branches were collected from a *M. intricata* colony living on the fringing reef of Mizugama, on the west coast of Okinawa island. It appeared that the colony had ampullae. The branches were kept in a glass bowl with seawater over night. The next morning three dead medusae were found in the bowl. Therefore, on the next day, I took several 4 - 5 cm fragments from the same colony and transported them to seawater beakers in the laboratory. About 20 individuals of medusae had been released at approximately 19:00 to 21:00, and about 24 individuals from 21:00 to 23:00. The released medusae swam with strong pulsations of the umbrella toward the surface of the water.

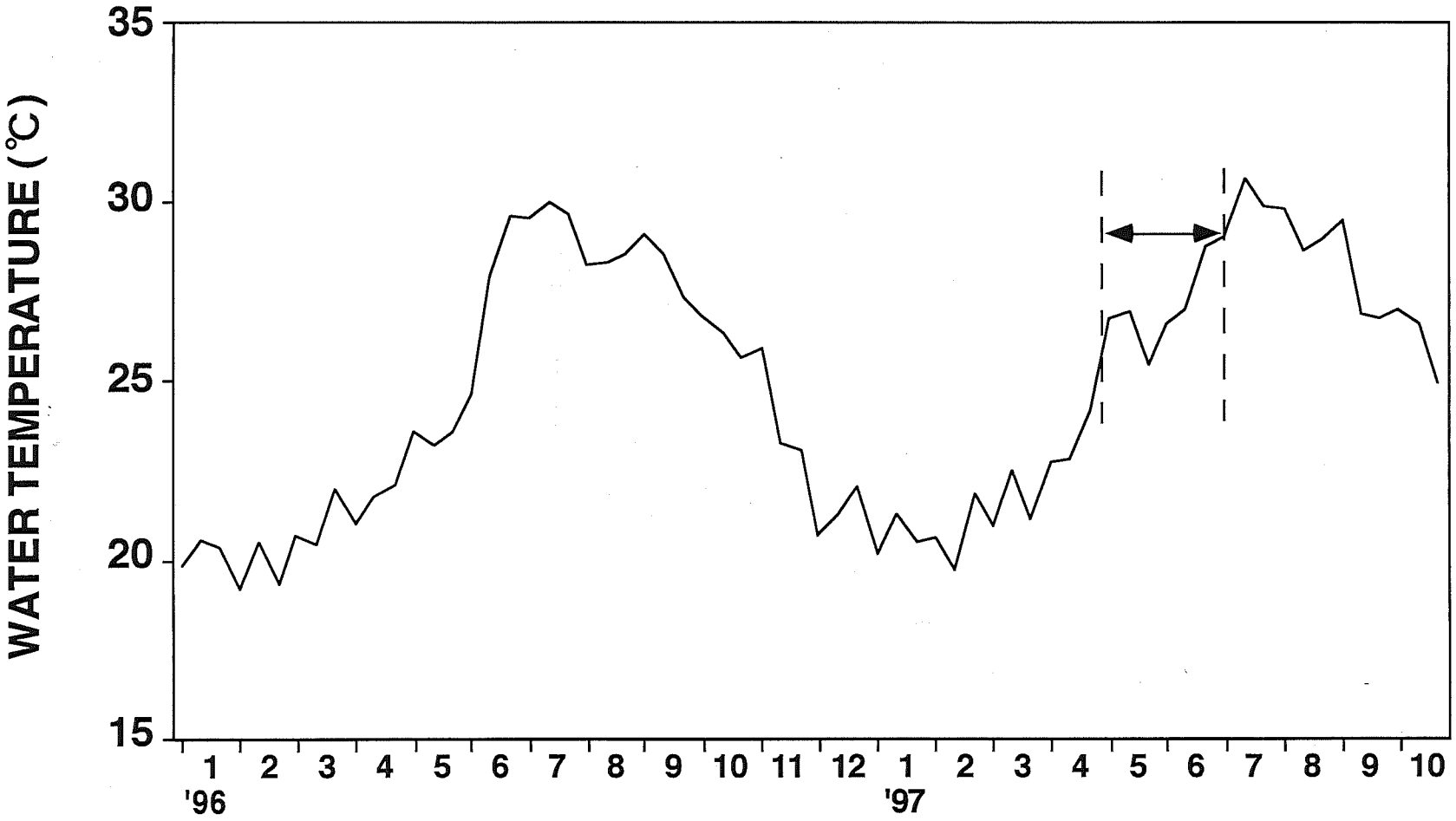


Figure 25. Mean sea surface temperature SST in front of TBRC (Tropical Biosphere Research Center). The medusa development was observed during May and June while the temperature was rapidly increasing.

The medusae were simple forms with neither tentacles nor marginal sense organs (Figure 26). The umbrella was about 1.0 mm in diameter with 3 or 4 clusters of nematocysts near the umbrella margin. Each medusa had a velum and narrow canals radiating from the bell margin, at the nematocyst clusters, to the apex of the umbrella (Figure 26). All the medusae had a rounded sperm sac at the top of the umbrella.

Dr. M. Yamaguchi collected some male and female medusae (Figure 27) from separate colonies of *M. intricata* in front of Tropical Biosphere Research Center (TBRC) on June 29, 1981. Most female medusae had 4 eggs, with many zooxanthellae (Figure 27b). He succeeded in obtaining fertilized eggs, and observed planulae on July 1, 1982. The planulae attached to the substratum on July 2, 1982 and transformed into calcifying spats with an initial diameter of about 0.8 mm.

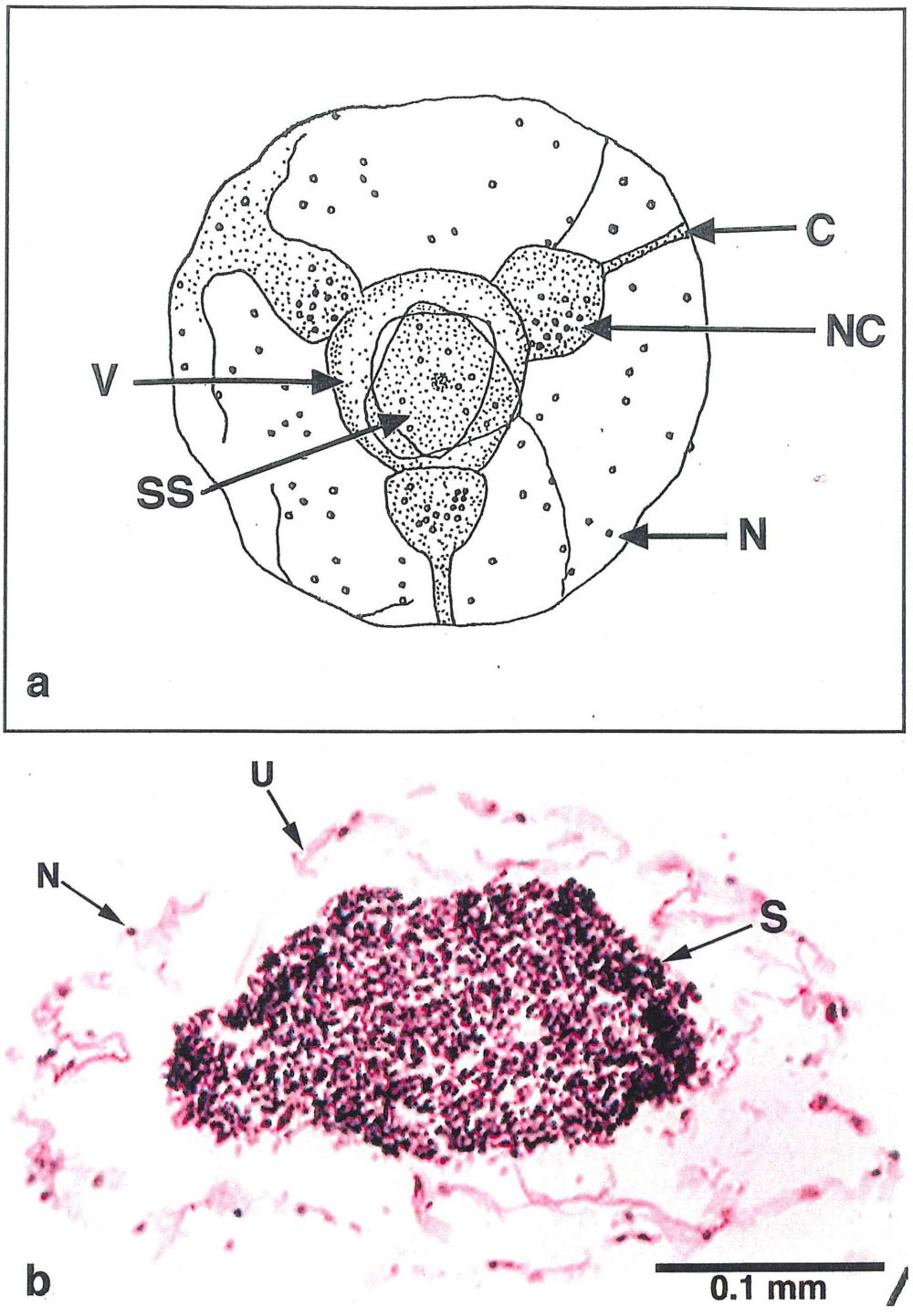


Figure 26. Released medusa of *M. intricata*. The samples were extracted from a *M. intricata* colony at Mizugama on 20 August 1996. **a)** Formalized medusa observed from the umbrella mouth. The specimen has been slightly shrunk. N: nematocyst, NC: Nematocysts cluster, SS: sperm sac, V: velum, C: canal. **b)** Histological section of the released medusa. U: umbrella, S: sperm.

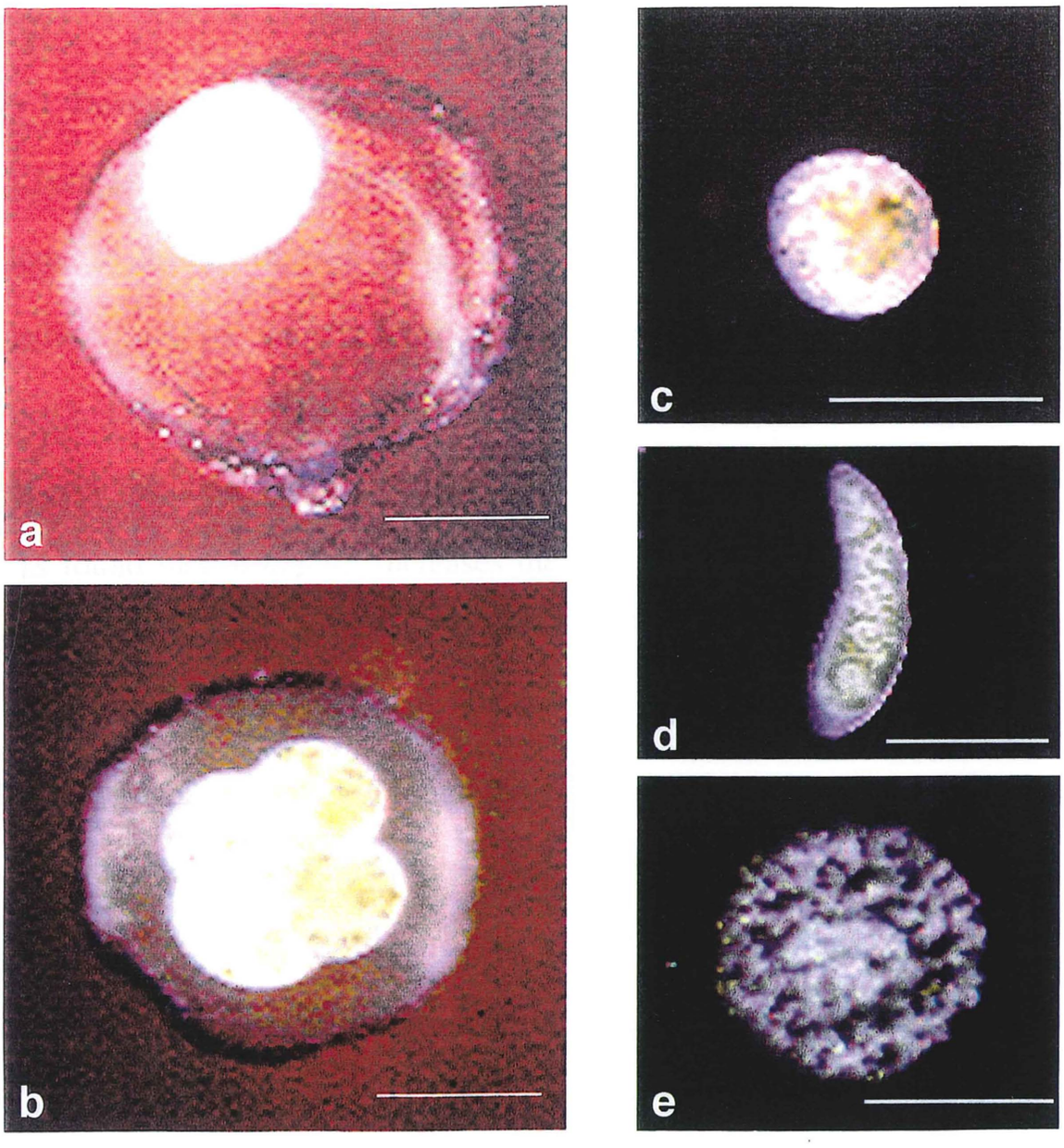


Figure 27. Medusae and early developmental stages of *M. intricata*. These photos were provided by Dr. M. Yamaguchi. All scale bars are 0.5 mm. **a)** Male medusa with sperm sac; **b)** Female medusa with four eggs in which many zooxanthellae are present; **c)** Egg with zooxanthellae; **d)** Planula; **e)** Spat attached to a substratum.