Chapter 12

A Developmental Study of the Japanese Medaka (*Oryzias Latipes*) and the Water Fern (*Marsilea*)

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247

247

Medaka

Introduction

The level at which the medaka eggs can be used is very broad. (The author has shown them to an enthusiastic fifth-grade class.) The medaka eggs are used in our 3-hour introductory biology laboratories, where the students have a limited knowledge of embryology. The emphasis is not on understanding the classical stages and movements of embryology, but rather, attention is directed toward observing how a vertebrate embryo is formed. They observe cleavage stages, and see groups of cells differentiate into tissues. At later stages, they can observe all the basic organs develop and still later see them begin to function.

These medaka eggs can be used in beginning, advanced and graduate courses in embryology and for research purposes.

The Japanese medaka is unsurpassed for use in descriptive embryological studies; it is far more suitable than live chick eggs, the usual material chosen. Its use is not restricted to embryological studies. The medaka and its eggs are also excellent material for physiological and genetic studies.

The advantages of the medaka for embryological studies have been listed in the booklet entitled, "The Japanese Medaka" by Robert V. Kirchen amd William R. West, © Carolina Biological Supply Company, Burlington, North Carolina.

"1. Breeding is controlled by photoperiodicity any time of the year.
2. Time of oviposition can be predicted so that the earliest developmental stages may be available for a given laboratory.
3. Eggs remain attached to the female for several hours after fertilization, during which time the cluster can be removed.
4. The adult mouth is such that only the larger fish in the aquarium can eat the smaller eggs; eggs are rarely eaten until they are brushed free of the female.
5. The egg is nearly transparent, except for oil globules in the yolk which soon coalesce at the vegetal pole away from the embryo proper.
6. Since the eggs can tolerate temperatures as low as 7°C, normal development can be slowed considerably to accommodate several laboratories."

Instructor's Materials

It is imperative for running this laboratory to send for the booklet "The Japanese Medaka" by R. V. Kirchen and W. R. West. It can be obtained from Carolina Biological Supply Company, Burlington, North Carolina 27215. This booklet contains all the information needed for setting up, breeding, and collecting eggs from medakas.

The time to begin preparation for this laboratory will depend on the time of year. The natural breeding season for the Japanese medaka is from mid-April to late September. For the rest of the year you will need at least ten days to initiate spawning by exposing the medakas to a daily photoperiod with a 16-hour light phase and an 8-hour dark phase. The time intervals can be arranged to provide freshly fertilized eggs at any given time. At 25°C it takes approximately 11 days after fertilization for hatching. This means that preparation for this laboratory should begin at least three weeks before the scheduled laboratory during the off-breeding season of the medakas.

The medaka eggs are collected daily and put into water-tight petri dishes filled with Embryo Rearing Medium (see booklet).

To increase the number of early stages for presentation, on the day of the lab, take the eggs fertilized that day, and divide them into groups. Leave some at room temperature and put some into ice water or a refrigerator (to 7°C) to slow down the development rate, or warm water (to 38°C) to increase the development rate.

At lab time place the medaka eggs on the lab benches in order of developmental stages. Remind the students to observe the early stages (fertilization through blastula) several times during the course of the laboratory so as to note the changes that occur. Try to have some medaka eggs hatch during lab time. This process fascinates the students. They are also interested in seeing what the parents look like.

Marsilea

Introduction

Marsilea, like the medaka, can be used in many ways, at many levels in the laboratory, from beginning biology students through students in advanced courses in plant biology. Simply nicking a sporocarp and placing it in water makes a dramatic demonstration in a lecture course, when discussing differentiation and development in plants. Studies can be made of the maturation of the mega-and microsporangia, of the male and female gametophyte, or of the developing embryo after fertilization.*

In this laboratory exercise emphasis is on comparing growth and development of a plant, from fertilization through establishment of a young sporophyte plant, with that of an animal from fertilization through hatching. Comparisons are also made with the life cycle of the angiosperm, *Lilium.*

*[Eds. note: See the chapter in this volume by Webb for additiomal information on methods and possible studies that can be done with *Marsilea.*]

Instructor's Materials

Preparation of *Marsilea* for this lab begins by nicking the coat of the sporocarps and placing them in a petri dish filled with aged water. The timing is crucial. At room temperature this procedure is done 14–16 hours before the scheduled laboratory period. At 30°C only 6 hours is needed for the male and female gametophytes to differentiate and produce mature eggs and sperm.

Different batches of sporocarps may vary as to maturation time. So it is an excellent idea, before the scheduled laboratory, to nick a sporocarp and follow with a dissecting microscope the time course of the maturation of the mega- and microsporangia. In this manner, you will learn to recognize the various stages. At the time of the scheduled lab, if sperm maturation is proceeding too quickly, simply cool down the mixture; if too slowly, apply heat carefully.

Within 10–15 minutes a transparent gelatinous wormlike structure begins to emerge from the sporocarp. This structure, called a sorophore (spore carrier), bears mega- and and microsporangia which are released into the water (see Figure 12.1). With the aid of a dissecting microscope, pick up megasporangia with a pasteur pipet and place in a separate petri dish. Make certain the dish is free of microsporangia. In the ensuing hours, the female gametophyte will develop within the megasporangium and the male gametophyte within the microsporangium.

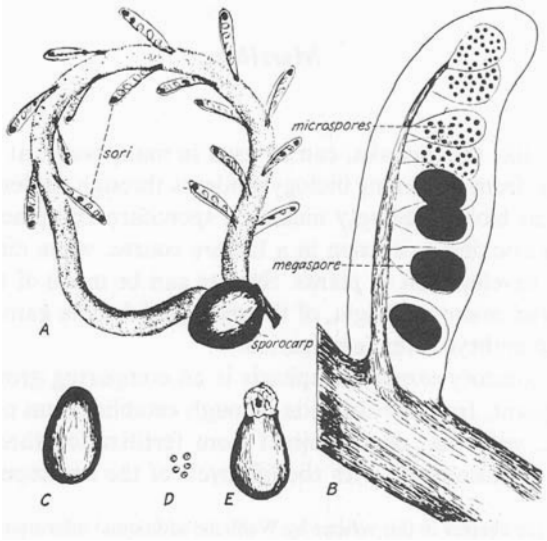

Figure 12.1. Sporocarp germination and spores in *Marsilea*. A, sporocarp with protuding gelatinous ring (sorophore) carrying sori; B, a sorus with megaspores and microspores; C, a megaspore; D, microspores; E, a megaspore germinating, with protruding female gametophyte. From Hill, J. B., Overholts, L. O. and Popp, H. W., Botany, a textbook for colleges, New York: McGraw-Hill Book Co., Inc., 1950. Reprinted with permission.

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As a demonstration, nick another sporocarp just as the laboratory session begins. This will enable students to recognize the various structures that they will see in the next part of the exercise.

Have the students proceed as follows: Carefully pipet up several of the separated megaspores and place them in the cavity of a concave slide. Cover with a coverslip held up with a few broken pieces of other coverslips. This is done to prevent the megaspores from being crushed. Place the slide under a compound microscope and, using high power, focus on the megaspores. Then, with a pasteur pipet, pick up a small amount of liquid containing sperm from the ruptured, matured microspores, and slowly and carefully place it under the edge of the coverslip. Look for rapidly swimming sperm and, with some luck, watch them swim to the megaspore, enter it, and fertilize the egg within. You can also see many swimming sperm getting stuck in the gelatinous sheath surrounding the megaspore (see Figure 12.2).

You will need a series of young embryos (young sporophyte ferns) from 24 hours after fertilization through several weeks or months of development. This can be accomplished by nicking a sporocarp and placing it in water at the appropriate interval before the laboratory.

Very few students have ever seen a mature water fern. They often ask what the young sporophytes will look like when they grow up. You can order mature plants of *Marsilea* from Carolina Biological Supply Company (see Figure 12.3).

Supplies and Materials

A. *Medaka*

Dissecting microscopes (Mag. 7 to 30X)
Dissecting lights for microscopes
Lens paper
A Medaka Breeding Set ($19.25) from Carolina Biological Supply Company, Burlington, North Carolina 27215
Medaka eggs (all stages from fertilization through hatching)
Medaka Booklets ($1.00 each; 10 or more, 75¢ each)

(N.B.: For large classes the expense of the Medaka Booklet may be prohibitive. Instead, you may order Carolina Tips, Vol. XXXII, #4, on Teleostean Development. It contains a series of 20 photographs of Medaka development. This pamphlet, while not as complete as the booklet, is suitable for an introductory course. The least expensive method of all is to make a series of simple drawings based on the photographs in the Medaka Booklet, accompanied by a brief description for each drawing.)

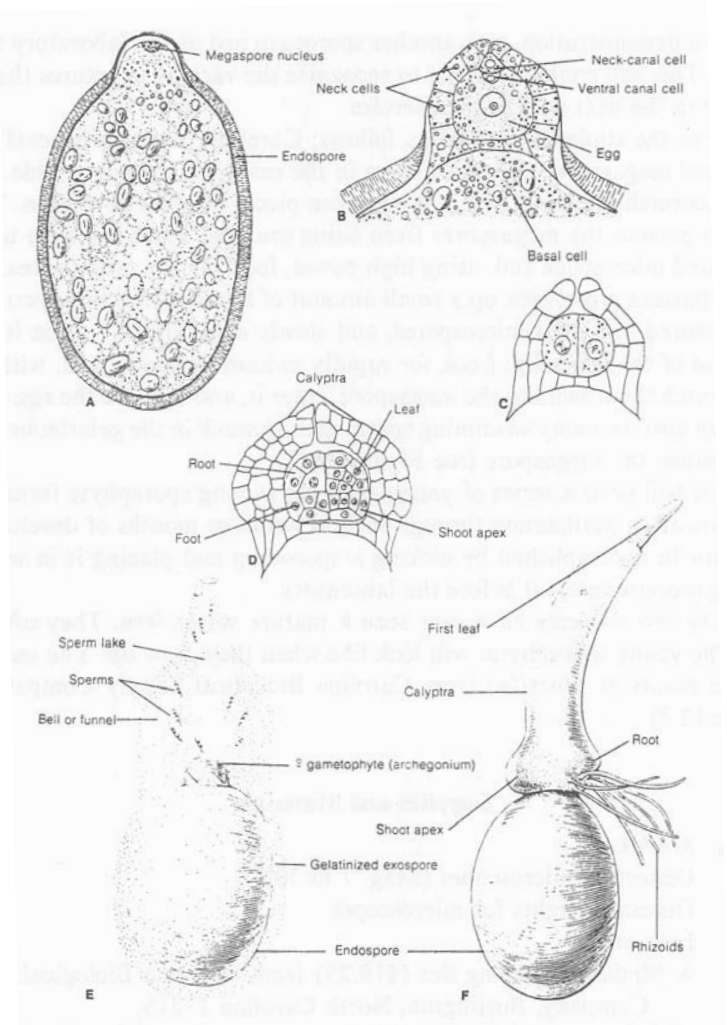

Figure 12.2 A-D *Marsilea quadrafolia.* A, longitudinal section of uninucleate megaspore, outer wall layer (exospore) removed; B, mature archegonium and portion of basal nutrative cell; C, two-celled embryo; D, embryos enclosed by developing calyptra. (A-D redrawn from Plant Morphology by A. W. Haupt, McGraw-Hill, 1953.) E-F *Marsilea vestita.* E, megaspore with enclosed megagametophyte surrrounded by gelatinous sheath in which sperms are embedded; F, external appearance of young sporophyte attached to megaspore and enclosed megagametophyte. From *Comparative Morphology of Vascular Plants,* Second Edition, by Adriance S. Foster and Ernest M. Gifford, Jr., W. H. Freeman and Company. Copyright © 1974.

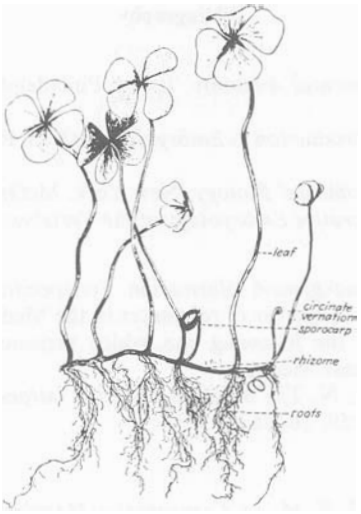

Figure 12.3. *Marsilea*, one of the heterosporous "water ferns." Drawings by Elsie M. McDougle. From Hill, J. B., Overholts, L. O. and Popp, H. W., Botany, a textbook for colleges, New York: McGraw-Hill Book Co., Inc., 1950. Reprinted with permission.

Aquarium with light source and timer
Embryo Rearing Medium (see Booklet)
Petri dishes (50 X 9mm with tight lid manufactured by Falcon)
Stainless steel forceps for egg removal
Fish net
Developmental Biology Filmloops (set of three) entitled, "Epiboly in the Killifish", $74.85, Kalmia Company, Concord, Mass. 01742.

B. *Marsilea*

Dissecting microscopes (Mag. 7 to 30X)
Dissecting lights for microscopes
Compound microscopes and light sources
Petri dishes (50 X 9 mm with tight lid manufactured by Falcon)
Slides with concave center and coverslips
Pasteur pipets with rubber tops
Marsilea sporocarps ($4.50) and mature plants from Carolina Biological Supply Company, Burlington, North Carolina 27215
Marsilea sporocarp for initial stages
Marsilea sporocarps grown at 30°C for six hours or at room temperature for 14–16 hours
Marsilea sporophytes at various stages from 24 hours after fertilization through several weeks or more of development

Bibliography

Medaka

1. Arey, L. B. *Developmental Anatomy.* 7th ed. Philadelphia: W. B. Saunders Co.; 1974.
2. Balinsky, B. I. *An Introduction to Embryology.* 4th ed. Philadelphia: W. B. Saunders Co.; 1975.
3. Berrill, N. J. *Developmental Biology.* New York: McGraw-Hill, Inc.; 1971.
4. Nelson, O. E. *Comparative Embryology of the Vertebrates.* New York: McGraw-Hill, Inc.; 1953.

The above give general background information. For specific detailed information on teleost development, look at the list of references in the Medaka Booklet. Included in that list of references is the following one, which presents a bibliography of 376 publications on the Japanese Medaka:

Briggs, J. C.; Egami, N. *The Medaka (Oryzias latipes).* A commentary and a bibliography. 1959, 16, 363.

Marsilea

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2. Haupt, A. W. *Plant Morphology.* New York: McGraw-Hill Book Co.; 1953.
3. Hill, J. B.; Overholts, L. O.; Popp, H. W. *Botany, A Textbook for Colleges.* 2nd ed. New York: McGraw-Hill Book Co.; 1950.