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# Laboratory Culture of the Lobate Ctenophore *Mnemiopsis mccradyi* with Notes on Feeding and Fecundity*

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*Abstract*

Collection and culture of the large lobate ctenophore *Mnemiopsis mccradyi* Mayer is described, including the requirements for successful development of larvae. Particular attention must be given to the collection of these delicate animals, the handling and provision of live microzooplankton of suitable size for the larvae, and the provision of food densities for the adults which neither stimulate "wasteful" feeding nor limit their growth. Although these ctenophores will ingest detritus and algal cells in high concentration, they lost weight at the same rate as starved individuals unless provided with living zooplankton. Under optimum conditions, specimens would lay eggs within 13 days of their own birth. By the 17th day they laid eggs daily, and had produced an average of 8,000 eggs within 23 days after birth. The maximum number of eggs laid by a single wild individual within 24 h after being brought into the laboratory was 10,000. Their high fecundity, rapid generation time, and ability to self-fertilize help to explain their sudden appearance in bloom proportions at periods of high food concentration in the environment, often referred to in the literature.

## *Introduction*

The phylum Ctenophora is exclusively marine and, with the exception of the order Platyctenea, entirely planktonic. Ctenophores have been reported from all oceans, and their occurrence in great swarms at certain times of the year in coastal and estuarine waters has been well documented (Bigelow, 1915; Nelson, 1925; Bigelow and Sears, 1939; Cronin *et al.*, 1962; Fraser, 1962). Concomitant with the appearance of ctenophores, a reduction in the numbers of other zooplankton (particularly copepods) has been observed, and has led a number of authors (Cronin *et al.*, 1962; Fraser, 1962; Hopkins, 1966) to speculate as to the possible importance of ctenophores in the population dynamics and productivity of marine plankton communities.

Despite these observations, little quantitative data on the seasonal abundance, ecology and life history of ctenophores exists, partly because very few workers have cultured or even maintained them in the laboratory. Greve (1970) and Hirota (1972) reported the culture of species of *Pleurobrachia* (order Cydippida, which retain their tentacles throughout life), but although several workers have made physiological measurements on lobate ctenophores (i.e., those belonging to the order Lobata, which lose their tentacles as adults) in the laboratory (e.g. Miller, 1970), they have not been raised over a life cycle. *Mnemiopsis mccradyi* Mayer occurs off the Gulf and Atlantic coasts of the United States, northwards to South Carolina. Baker (1973) reported on its population dynamics in Biscayne Bay, Miami, and Reeve and Baker (in press) estimated the production of this population from laboratory growth-rate studies.

## *Collection, Handling and Rearing*

*Mnemiopsis mccradyi* reaches a much larger size than species previously cultured, i.e., approximately 70 mm long and 300 mg ash-free dry weight compared with 15 mm in diameter and 20 mg for *Pleurobrachia bachei* (Hirota, 1973). This, combined with their less compact shape and greater fragility (unlike *P. bachei* they rapidly disintegrate in standard preservatives) makes them extremely susceptible to damage and fragmentation during collection procedures.

Specimens were collected by slow towing (1 to 2 knots) at the surface for 2 to 3 min or less, with a 1 m mouth diameter net of 705 µm mesh fitted with a flexible vinyl 14 1 cod end. The large capacity of the collection bag, as well as its flexibility, minimized physical damage. As a further precaution against injury, the ctenophores were picked out of the top of the collection bag using a small glass beaker, and transferred to plastic buckets containing seawater. The col-

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lections were usually returned to the laboratory within 15 min, when the ctenophores were transferred by glass beakers to aquaria containing 30 l of seawater. The aquaria were aerated very gently with air diffusers, since excessive bubbling results in injury to these delicate animals.

Usually within 12 h of being brought into the laboratory, these hermaphroditic animals would produce eggs, which were visible in the aquaria as a fine haze of tiny particles. The eggs are spherical, 0.12 to 0.22 mm in diameter, and are encased in a thin membranous capsule 0.38 to 0.52 mm in diameter. Egg development was rapid and, within 12 to 20 h at either 21°, 26° or 31°C, larvae hatched from the capsules. The larvae are 0.28 to 0.32 mm in diameter and resemble members of the order Cydippida.

The eggs and larvae are very sensitive, and highest survival was obtained if they were handled minimally over the first few days. Greve (1970) reported less than 1% survival of *Pleurobrachia pileus* larvae transferred to larger tanks after hatching. Likewise, attempts at transferring larvae of *Mnemiopsis mccradyi* to fresh tanks either by gentle siphoning with 15 mm diameter tubing or direct transfer in 2 l beakers resulted in very low or no survival. However, when adult ctenophores were removed and larvae held in the tanks in which they had hatched, 30% survival was recorded on the fifth day.

Larvae were raised in batch cultures which were maintained and fed in the manner described by Reeve and Walter (1972) for the chaetognath *Sagitta hispida*, since in terms of initial size, sensitivity to mechanical damage, and requirements for food consisting of natural living zooplankton of size progressively increasing from that which would pass through a 100 µm mesh, they were closely similar.

Larvae fed by seining food organisms from the water with their outspread tentacles (see Rowe, 1971). The oral lobes took over the feeding function as the tentacles were lost (15 mm and larger) by creating ciliary water currents, from which food organisms were entangled in mucous strands and transferred into the stomodeum. At high food concentrations, the lobate adults tended to accumulate a mucous food bolus very rapidly and either not ingest part of it or, if ingested, to sometimes reject it. This behavior resulted in high mortality of the zooplankton without utilization by the ctenophore. The undigested food accumulated on the bottom of the tanks and, at times, resulted in fouling of the tanks and death of the ctenophore population. In order to avoid this, the number of ctenophores maintained in each tank was reduced to 25 individuals per tank when they reached 15 mm, and food was provided in smaller quantities at more frequent intervals (daily or twice daily as required). Growth rates of cultured *Mnemiopsis mccradyi* at various temperatures was documented by Baker (1973) and Reeve and Baker (in press). Three generations of ctenophores were raised in the laboratory from an initial wild parent.

*Food Preferences*

Ctenophores are generally considered to be carnivores which feed on a variety of other zooplankton such as copepods, mollusc veligers, barnacle nauplii, etc., and ctenophores reared on natural zooplankton grew rapidly. However, the possibility that ctenophores utilize other sources of food, such as detritus, nannoplankton, or phytoplankton, has been suggested by Nelson (1925) and Miller (1970). In order to investigate whether *Mnemiopsis mccradyi* was able to ingest and utilize food sources other than natural zooplankton, some feeding experiments with algal suspensions and particulate detritus were carried out.

The particulate detritus used in these experiments consisted of both recognizable plant and animal material which was collected in plankton nets. In the laboratory, the plankton collections were passed through a 500 µm mesh to remove larger zooplankton (medusae, decapod larvae) and allowed to settle for about 15 min, during which time the majority of the live zooplankton congregated on the surface. The water containing the zooplankters was then decanted, and the remaining debris was dried in an oven at 60° C and used in the feeding experiments as required. The dried detritus was examined under a microscope to determine its composition and the size range of the particles. The detritus consisted primarily of plant material (pieces of algae, seagrasses, mangrove peat) but some animal material (copepods, veligers, chaetognaths, etc.) was also present, and the particles ranged in size from 0.05 to 2.0 mm, in their longest dimension.

In preliminary experiments, ctenophores which had not been fed for 24 h were placed individually in plastic jars containing 200 ml of either an algal (*Chlorella* sp. at 600,000 cells/ml, *Rhodomonas baltica* at 100,000 cells/ml) or detrital suspension for a period of 2 h. The ctenophores were then transferred to glass crystallizing dishes, examined under a dissecting microscope, and the presence or absence of detritus and algae noted. Each feeding group consisted of 26 specimens, ranging in length from 4.6 to 20.0 mm. At the end of the 2 h feeding interval some observable "food" was present in the stomadeum of 42% of the "*Chlorella*" group, 63% of the "*Rhodomonas*" group, and 73% of the "detritus" group. However, in all three groups, the "food" did not appear to be appreciably concentrated within the stomadeum of the ctenophores, and in the case of the two algal groups it appeared more as a fine haze of particles similar in density to that of the medium, which could possibly have been the result of simple ingestion of fluid.

Nevertheless, in view of the fact that these food-stuffs were observed in the stomadeum of the ctenophores, the possibility that they were utilized by them could not be ruled out, and subsequent experiments were performed to inves-

tigate whether algal or detrital suspensions could sustain or promote growth of the ctenophores.

In the first experiment, 48 larval ctenophores (2.0 to 2.4 mm in length) were divided into 8 groups of 6 ctenophores each and placed in 2 l beakers containing 1,600 ml of membrane-filtered seawater. Four feeding regimes, each consisting of 2 groups of 6 individuals were employed. The feeding regimes were: (1) *Phaeodactylum tricornutum*, 100,000 cells/ml; (2) *Chlamydomonas coccoides*, 100,000 cells/ml; (3) natural zooplankton (50 to 100 µm fraction), consisting primarily of copepod nauplii; (4) no food. The ctenophores were transferred daily to new food supply and water. The experiments were terminated on the 5th day, and the length of the ctenophores was measured.

The second experiment consisted of 3 groups of 12 ctenophores (15.4 to 17.5 mm in length) held in aquaria containing 40 l of filtered seawater. Added to the aquaria were either: (1) natural zooplankton (200 to 300 µm fraction), consisting primarily of adult *Acartia tonsa* copepods; (2) particulate detritus as previously described; (3) no food. This experiment was terminated on the 7th day. Food was added daily, and half of the volume of water was removed and renewed on the 3rd and 5th days.

The third experiment was similar to the second except that, instead of the detrital group, 1 group of ctenophores was fed a mixture of algae (*Phaeodactylum tricornutum, Dunaliella varians,* and *Monochrysis* spp.) maintained at a concentration of approximately 100,000 cells/ml.

The size of the ctenophores at the beginning and end of each experiment is presented in Table 1. In all three experiments, the ctenophores fed natural zooplankton showed a substantial increase in size, whereas the ctenophores fed on either detrital or algal suspensions decreased in size to an extend similar to that recorded for unfed individuals. The lack of any growth response and the actual decrease in size of the ctenophores fed selected algal species or detritus suggests that the ctenophores are unable to utilize either of these food sources to promote growth or even meet their energy requirements.

Table 1. *Mnemiopsis mccradyi*. Effect of various foods on maintenance and growth

| Experiment no. | Food type | Mean length (mm) | | Change in length (%) | Duration of experiment (days) | Number of specimens |
|---|---|---|---|---|---|---|
| | | At start | At end | | | |
| 1 | *Phaeodactylum tricornutum* | 2.10 | 1.47 | -30 | 4 | 6 |
| | *Phaeodactylum tricornutum* | 2.30 | 1.84 | -20 | 4 | 6 |
| | *Chlamydomonas coccoides* | 2.29 | 1.80 | -21 | 4 | 6 |
| | *Chlamydomonas coccoides* | 2.28 | 1.69 | -26 | 4 | 6 |
| | Natural zooplankton | 2.09 | 5.78 | +177 | 4 | 6 |
| | Natural zooplankton | 2.41 | 5.59 | +132 | 4 | 6 |
| | No food | 2.20 | 1.70 | -23 | 4 | 6 |
| | No food | 2.15 | 1.62 | -25 | 4 | 6 |
| 2 | Particulate detritus | 15.4 | 10.4 | -32 | 7 | 12 |
| | Natural zooplankton | 17.5 | 32.9 | +88 | 7 | 12 |
| | No food | 16.2 | 10.1 | -38 | 7 | 12 |
| 3 | Mixed algae[a] | 21.9 | 15.9 | -27 | 8 | 7 |
| | Natural zooplankton | 19.4 | 37.8 | +95 | 8 | 7 |
| | No food | 21.5 | 13.0 | -40 | 8 | 7 |

[a] *Phaeodactylum tricornutum, Dunaliella varians, Monochrysis* sp.

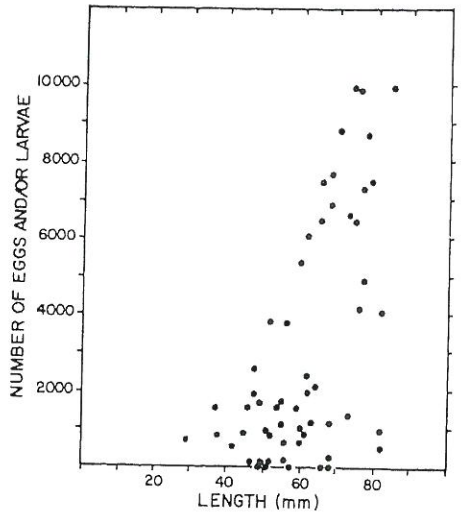


Fig. 1. *Mnemiopsis mccradyi*. Egg and/or larvae production, as function of size, over 24 h following collection of ctenophores from the field

*Fecundity*

Information on egg production was obtained for both field-collected and laboratory-reared ctenophores. Approximately 60 field-collected ctenophores were placed individually in 2 l containers of seawater. On the following morning the number of larvae and/or eggs were estimated by counting 6 aliquots of the total volume, and the length of the adult was measured. Only 6 specimens failed to produce offspring, and the numbers produced by the rest ranged from 169 to 9990. There was wide variation in egg production for a given size of ctenophore, but the maximum egg production was proportional to increasing size (Fig. 1). These observations suggested that egg production can occur over an extensive portion of the lifespan of *Mnemiopsis mccradyi*, as indicated by the size range (38 to 85 mm in length) of individuals which produced eggs, and is most probably a continuous process, dependent on the state of nutrition of the ctenophores.

In order to obtain an estimate of the minimum size at which reproduction could occur, as well as the possible frequency of reproduction, laboratory-reared individuals of known age were examined for egg production. Six ctenophores originally begun in batch culture were removed on the 6th day at a size range of 3.6 to 5.4 mm in length, and placed individually in aquaria containing 19 l filtered seawater. Each day they were removed from the tanks, and the contents of the tanks concentrated to a 1 l volume by slow siphoning with an 8.5 cm diameter funnel fitted with a 50 µm mesh to retain the eggs and/or larvae. The concentrate was gently poured into a 2 l beaker, and the eggs and/or larvae counted. The ctenophores were transferred into filtered seawater and food added. This procedure was repeated until the 23rd day after hatching, when the experiment was terminated.

The numbers of eggs and/or larvae produced and the increase in length for each of the 6 individuals are presented in Table 2. All 6 ctenophores first produced eggs on the 13th day after hatching. The minimum length at which eggs were first produced was 29 to 34 mm. The number of eggs produced at this time was low, ranging from 7 to 147 eggs/individual. Reproduction was intermittent over the next 3 days, and 4 of the 6 individuals did not produce any eggs during this period. However, on the 17th day, when the ctenophores ranged in size from 42 to 60 mm, egg production was resumed by all 6 individuals and continued thereafter on a daily basis until the 23rd day, when the experiment was terminated. The total number of eggs produced by the ctenophores over the 17 day period ranged from 5,690 to 12,423 eggs/individual, with an overall average of 8,210 eggs/individual. The number of eggs produced by an individual varied on a day-to-day basis, but without any apparent pattern. The average daily egg production of the 6 ctenophores ranged from 517 to 1,130 eggs/individual/day, with a mean value of 746 eggs/individual/day. However, if only the sustained period of egg production (17 to 23 days after hatching) is considered, the mean daily production would increase to 1,173 eggs/individual/day.

Daily egg-production rates in these cultured specimens never reached the maximum numbers produced overnight by field-collected individuals of comparable size. Whether the trauma of removal from the natural environment caused release of more eggs than would have occurred had they been left undisturbed over that period, whether those numbers could have been sustained over subsequent days as in the laboratory-reared specimens, or whether the ctenophores from the field had been exposed to higher food levels, is not known. In any case, only about one-third of the field-collected ctenophores laid more than 2,000 eggs over 26 h.

Egg production of the cydippid ctenophore *Pleurobrachia bachei* reared in the laboratory has been followed by Hirota (1972). He also observed two periods of egg production in this smaller tentaculate species, an early period beginning at about 2 mm in diameter during which only a small number (80) of eggs and/or larvae are produced, followed by a period of sustained (daily) reproduction in mature adults beginning at about 8 mm in diameter; the two periods were often separated by a period of no egg production.

The average number of eggs reported by Hirota (1972) for adult *Pleurobachia bachei* was 350 eggs/individual/day, which is some two to three times lower than the production of eggs obtained for laboratory-reared *Mnemiopsis mccradyi* in the present study. In comparison with other zooplankton, the number of eggs produced both by *M. mccradyi* and *P. bachei* are quite high. For the chaetognath *Sagitta hispida*, over a 20-day period,

Table 2. *Mnemiopsis mccradyi*. Daily length increments and egg production for 6 ctenophores reared individually in the laboratory

| Days after hatching | Ctenophore number 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Length (mm) | Eggs (no.) | Length (mm) | Eggs (no.) | Length (mm) | Eggs (no.) | Length (mm) | Eggs (no.) | Length (mm) | Eggs (no.) | Length (mm) | Eggs (no.) |
| 6 | 4.64 | - | 5.44 | - | 3.68 | - | 4.48 | - | 3.84 | - | 4.48 | - |
| 7 | | - | | - | | - | | - | | - | | - |
| 8 | | - | | - | | - | | - | | - | | - |
| 9 | 14.4 | - | 12.0 | - | 12.5 | - | 13.4 | - | 12.0 | - | 12.3 | - |
| 10 | | - | | - | | - | | - | | - | | - |
| 11 | | - | | - | | - | | - | | - | | - |
| 12 | 28.0 | - | 23.0 | - | 26.0 | - | 27.0 | - | 31.0 | - | 31.0 | - |
| 13 | 32.0 | 57 | 29.0 | 77 | 33.0 | 58 | 31.0 | 14 | 34.0 | 7 | 34.0 | 147 |
| 14 | 39.0 | - | 34.5 | 76 | 39.0 | - | 38.0 | - | 40.0 | - | 38.0 | - |
| 15 | 42.0 | - | 39.0 | 44 | 45.0 | - | 43.0 | - | 45.0 | - | 44.0 | - |
| 16 | 46.5 | 188 | 40.0 | - | 53.0 | - | 47.0 | - | 46.0 | - | 46.0 | - |
| 17 | 50.0 | 935 | 42.0 | 61 | 60.0 | 387 | 52.0 | 273 | 46.0 | 248 | 49.0 | 290 |
| 18 | 53.5 | 912 | 48.0 | 146 | 65.5 | 1792 | 56.5 | 310 | 48.0 | 361 | 51.0 | 305 |
| 19 | 55.0 | 1906 | 48.0 | 1016 | 67.0 | 1617 | 60.0 | 1915 | 49.0 | 917 | 56.0 | 1528 |
| 20 | 59.0 | 2514 | 50.0 | 1154 | 74.0 | 2120 | 61.0 | 1885 | 50.0 | 1800 | 58.0 | 926 |
| 21 | 61.0 | 1917 | 53.0 | 780 | 76.0 | 1927 | 62.0 | 1293 | 51.0 | 464 | 58.0 | 490 |
| 22 | 62.0 | 2064 | 58.0 | 1067 | 78.0 | 1153 | 65.0 | 1068 | 53.0 | 1101 | 59.0 | 1047 |
| 23 | 64.5 | 1930 | 58.5 | 1691 | 80.0 | 1186 | 66.0 | 1976 | 54.0 | 1158 | 61.0 | 957 |
| Total | | 12423 | | 6112 | | 10240 | | 8734 | | 6056 | | 5690 |

Reeve (1970) reported a maximum of 420 eggs/individual. A mean of 2,000 eggs was produced by the copepod *Calanus helgolandicus* over a 50-day period (Paffenhöffer, 1970), and Ponomareva (1959) estimated that *Euphausia pacifica* produced 1,400 eggs over its life span.

One further point concerning the reproductive biology of ctenophores is that the production of fully developed larvae as well as eggs by individually-reared *Mnemiopsis mccradyi* isolated as early as 3.68 mm in length, demonstrated that these ctenophores are capable of self-fertilization. Hirota (1972) also reported self-fertilization for individually-reared *Pleurobachia bachei*. In terms of the ecology of the ctenophores, the high fecundity of these hermaphroditic animals coupled with the ability for self-fertilization are most probably important factors in their ability to build up large local populations under favorable conditions of food supply. This must be especially true of warm-water lobate ctenophores such as *M. mccradyi*, which can attain 300 mg ash-free dry weight within 40 days compared to 20 mg in 100 days for *P. bachei* (Hirota, 1973).

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