Browne Lab

Light-Dark Cycle protocol for spawning warm water *Mnemiopsis*

This protocol is modified from Pang *et al.* 2008 and works well for our animals from warm coastal waters surrounding South Florida. It has been reliable for spawning freshly caught adults as well as longterm captive adults. All steps are completed at room temperature (~21-23°C).

1. Place individual *Mnemiopsis* adults in round glass culture dishes (4 inch diameter for small animals, 8 inch diameter dish for large adults).

2. Remove as much seawater as possible and refill with clean seawater (either filtered natural seawater or artificial seawater). Remove remaining debris and mucus with a pipette. Repeat at 5-10min intervals 2-3x.

3. Prior to placing adults in the dark it is useful to screen the gonads for mature gametes. Presence of mature oocytes and sperm will identify animals with the highest fecundity.

Freeman and Reynolds, 1973

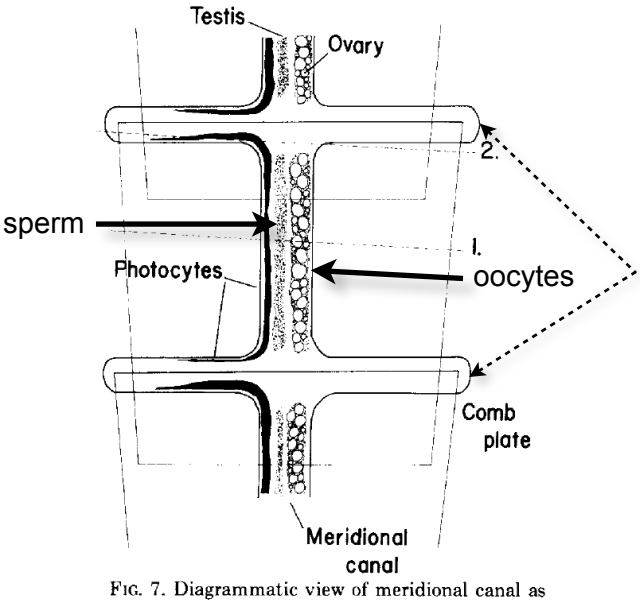
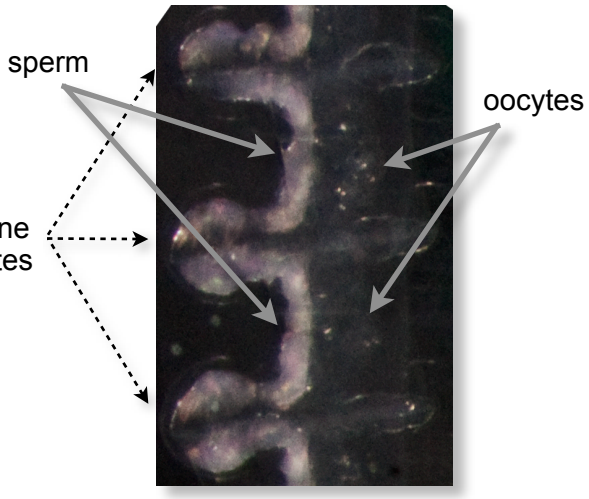


FIG. 7. Diagrammatic view of meridional canal as seen from above. The aboral end of the canal is up. The relative position of the photocytes, the testes, and the ovaries are indicated. *1.* indicates the approximate position of a cross-sectional view of the canal shown in Fig. 8. 2. indicates the approximate position of the cross-sectional view of the canal shown in Fig. 9.



Adult *Mnemiopsis* meridional canal. Aboral is up. Mature sperm is readily visible as opaque thickenings flanking the left side of the canal. Mature oocytes are visible as clumps of translucent round material flanking the right side of the canal. Gametes are released *en masse* through pores associated with the ctene plates. Sperm release typically precedes oocyte release.

4. Place dishes with animals in <u>complete darkness and do not disturb</u> for ~3 hours.

5. After 3 hours remove *Mnemiopsis* adults from the dark. Pulses of synchronous spawning should begin within 1.5-2.5 hrs after re-exposure to light. Monitor animals at ~15-20min intervals for signs of gamete release. Continue removing mucus/debris as needed.

   Typically sperm are released into the water column prior to oocyte release. High sperm concentration (cloudy water) can lead to abnormal embryonic development (polyspermy). If the water becomes very cloudy during sperm release, remove a significant proportion and replace with clean seawater (make sure you aren't also removing freshly released oocytes!).

6. After spawning adults can be gently transferred by hand to either a new dish or back into a longterm culture tank.

7. Allow eggs to settle for a few minutes. A pipette can be used to collect embryos from the bottom of the dish. If sperm is still present at high concentrations (cloudy water), transfer the freshly fertilized embryos to a dish with clean seawater. Continue incubation of embryos at RT to the desired developmental stage.

Notes:
- *Mnemiopsis* adults can be spawned multiple times if given sufficient time to recover and also fed heavily between spawning cycles (a few days), however the highest yields of synchronous embryos are consistently obtained from freshly caught wild animals.
- Animals captured on cloudy/overcast days often exhibit lower spawning success.
- Animals captured from *very* warm waters (>33°C) appear to have arrested oogenesis.

References:

Freeman, G., Reynolds, G.T.. 1973. The development of bioluminescence in the ctenophore *Mnemiopsis leidyi*. Developmental Biology 31: 61-100.

Pang, K., Martindale, M.Q.. 2008. *Mnemiopsis leidyi* spawning and embryo collection. Cold Spring Harbor Protocols doi:10.1101/pbd.prot5085.