

**The Ultimate *Capitella teleta* Culturing Protocol\***

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**I. Set up**

- **WARNING: DO NOT USE DETERGENTS/CHEMICALS ON ANY ANIMAL-SAFE EQUIPMENT OR MATERIALS!!!**
- Our *Capitella* cultures are kept at two different temperatures: 15°C and 19°C.
  - ❖ The animals will develop faster at the higher temperature, but produce smaller broods.
  - ❖ We shift them to the appropriate temperature according to current experimental needs.
- The worms are kept in glass finger bowls (35-40 worms/bowl).
  - ❖ Dimensions of bowl: depth=1 1/2" (3.8 cm); diameter=10.2 cm (4").
  - ❖ We condition the bowls (and other animal-safe equipment) before using them for the first time, by letting them sit in the seawater tanks for 2-3 days.
  - ❖ Two or three bowls are stacked and the top bowl is covered with one side of a 150mm x 15mm Petri dish. (Make sure that water between the bowls/lid does not form an air-tight seal, and that there is a layer of air (1/2-1 in) between the top of the water and the top of the bowl. *Capitella* are sensitive to oxygen availability.)
- The bowls are filled approximately halfway with 20 µm filtered seawater (SW).
  - ❖ This SW is obtained from the Whitney Sea Table room and is naturally filtered. Therefore, no additional filtration is needed.
  - ❖ Periodically (once every 1-2 months or if you notice a slightly green/black tinge), the carboy and squirt bottles must be rinsed and scrubbed thoroughly with hot water and several seawater rinses to prevent build up of algae and other microbes. **DO NOT USE DETERGENTS/CHEMICALS ON ANY ANIMAL-SAFE EQUIPMENT OR MATERIALS.** If tap water is used on any bowls/equipment, be sure to give it a rinse with SW or de-ionized water (diH<sub>2</sub>O) before using.
  - ❖ At Whitney, we generally do not need to worry about the salinity of the SW.
    - However, if you notice that a lot of seawater has evaporated, the salinity is probably higher than normal. Slowly bring water level back to normal by adding a little bit of SW at a time, and waiting 20 minutes in-between to allow the animals time to readjust. The shock of an abrupt change in salinity can kill them → several dead animals in a bowl may mean that there was a sudden salinity change!
    - Salinity should be maintained at 32 ppt. or 1.023-1.025 specific gravity (can be measured with a hydrometer – available at pet stores). If using conditioned artificial seawater, filter before adding to finger bowls, check salinity and adjust with diH<sub>2</sub>O – slowly! – if necessary. You can make readjustments by checking the salinity of the old water with a hydrometer and then coming close to that.
    - Recent evidence suggests that *Capitella* may actually do better at salinities as low as 20-25ppt. For more information see: Pechenik, J.A., Berard, R. and L. Kerr (2000). Effects of reduced salinity on survival, growth, reproductive success, and energetics of the euryhaline polychaete *Capitella* sp. I. J. Exp. Mar. Biol. Ecol. 254: 19-35.

**II. Mud Incubation Protocol**

- The worms are cultured in mud, which serves as the substrate through which they burrow and the food source. They ingest mud particles and detritus, and extract organic nutrients from the bacteria in the mud.
  - ❖ The dirt on the dirt
    - Collect “high quality” mud from a (saltwater) mudflat: mud, not just sand.
    - Sift the mud over a #30 mesh (0.6mm) sieve to remove the “big stuff” (use SW when sifting).
    - Aliquot the sifted mud into Tupperware containers. This way if the mud goes “bad” in one container you have many back-ups.

- Thoroughly freeze the mud (for at least 3 days) at  $-20^{\circ}\text{C}$  to kill any large organisms that may be in the mud.
- Keep the aliquots frozen until needed.
- Mud Quality: The nutritional quality of the mud can often be poor when taken straight from the field. To enrich the mud, we now do a “**Mud Incubation.**”

**Mud Incubation:**

- Defrost pre-sieved mud. (Recommended:  $\sim 1/2$  small plastic bucket)
- Place in bucket and add about half a cup of powdered kelp (too much kelp is bad!), a small amount of re-suspended frozen algae, and a small spoonful of post-sifted mud from the large glass bowl (for inoculation) and mix thoroughly.
- Make sure  $\sim 1$  inch of SW covers the mud (don't want it to dry out!)
- Cover bucket loosely (we use a metal stove top cover).
- In 3-5 days, check to see that mud has been inoculated (should start “bubbling” and have a strong odor).
- Let incubate at room temperature for  $\sim 4$  weeks. (This is the time it takes for the mud to shift from the anaerobic phase [different bacterial strains are toxic to worms!] to the aerobic phase i.e. the peak of the bacterial growth.)
- To test if the mud is ready to feed to the worms, gently place a small spoonful in finger bowl with worms. Leave undisturbed for 1-2 days, then check to see if any worms have burrowed into it (see indications of activity e. g. tubes).
- At this point, the mud should be transferred to airtight containers and stored at  $4^{\circ}\text{C}$  ( $-20^{\circ}\text{C}$  for long-term storage), before the bacterial levels begin to decline.
- In addition, try to keep mud covered with a layer of SW and from being exposed to too much oxygen, which also depletes the bacteria (e.g. keep lid closed when not in use).
- If the mud still seems too strong (has an acrid smell), you can cut it half-and-half with older/non-incubated mud.
- Store the current working container at  $4^{\circ}\text{C}$ .
- Smell the mud each time it is used to check the quality. *The ‘stinkier’ the better!*

### III. Feeding

- ❖ For the first week after starting new bowls, just change the SW.
- ❖ For all bowls older than one week: feed and change the SW once a week.
  - Gently decant off excess water from the finger bowl.
  - Add mud.
    - a) For bowls  $\geq 2$  months of age, 1 tablespoon of mud
    - b) For bowls  $< 2$  months of age,  $1/2$  tablespoon of mud
  - Add supplemental tetraamin fish flakes that are ground up very fine
    - a) For younger worms  $< 1$  month, volume:  $50\mu\text{l}$  crushed flakes/bowl
    - b) For mature worms  $> 1$  month,  $100\mu\text{l}$  crushed flakes /bowl
  - Fill the bowl to approximately 1 inch below the top with SW (this should leave a sufficient air space between the water and lid/other bowl). If the water level is close to the top, even if it does not initially form a seal, when bowls get moved water will slosh up and form a seal.
    - Good to add the water such that the mud is temporarily resuspended  $\rightarrow$  aerates the mud
- The quality and quantity of the food matters is important. The better fed they are, the faster they grow and reproduce. (We have a number of articles on this subject if you want to read up on it.) If you notice that the worms are getting smaller and smaller, this is ALMOST ALWAYS a problem with the poor quality of the mud.

- OPTIONAL CULTURING: If quality of mud is in question or in limited quantity, worms can be grown in sand with tetramin flakes added as a nutritional supplement.
  - ❖ Try to collect finest sand possible
  - ❖ Freeze sand at  $-20^{\circ}\text{C}$  for at least 3 days to kill outside critters
  - ❖ Thaw and aliquot into finger bowls approximately 0.5 cm to cover entire bottom of bowl
  - ❖ Stir in 50 $\mu\text{l}$  crushed tetramin flakes
  - ❖ Fill with SW and add 40 larvae.
  - ❖ Maintain bowls by changing SW and adding 50 $\mu\text{l}$  fresh tetramin flakes 1x/week.
  - ❖ Once the bowls are  $\geq 1$  month, add 100 $\mu\text{l}$  crushed tetramin flakes instead of 50 $\mu\text{l}$ .

#### IV. Sifting

- Each bowl of adult (i.e. sexually mature) worms must be sifted every two weeks to prevent larvae from undergoing metamorphosis in the bowls. If this happens, the number of individuals in each bowl can quickly become hundreds of individuals, crowding the bowl and is suboptimal for growth rates.
  - ❖ Set up a new, clean finger bowl with fresh mud, tetramin flakes and SW.
  - ❖ Gently pour off the water from the bowl to be sifted.
  - ❖ Using squirt bottle filled with SW, squirt the contents of the bowl (mud and worms) onto a 1mm sieve, which is held over a large glass discard bowl to catch the mud falling through – continue squirting until all of the mud has run through (water runs clear) and only worms (and some larger pieces of sand/debris) are left on the sieve. Be gentle squirting the SW so you don't damage the worms.
    - If juveniles are to be sifted, use a 150 $\mu\text{m}$  sieve.
  - ❖ Turn sieve upside down and squirt the worms into the bottom half of a 150mm x 15mm Petri dish (prewet bottom with SW). (Note: Do not delay between squirting worms off the sieve into the Petri dish, otherwise worms will “weave” themselves in the mesh and it is difficult to get them out.)
  - ❖ Using a dissecting microscope and a pair of forceps:
    - Set aside any brood tubes (for starting new bowls, dissecting, etc.).
    - Remove any unhealthy and/or old worms (when the worms get old or are unhealthy, they turn yellow/brownish and produce smaller, abnormal broods/larvae). Some worms may also not grow to full size potential and they abnormally small worms should also be discarded. If need be, two bowls of a similar age can be combined into one as some worms start to die or become unhealthy.
    - Transfer the remaining worms to the new bowl.
    - Remember to maintain a density of  $<40$  worms/bowl. If there are too many worms in a single bowl, they compete for food and their growth gets stunted (e. g. they will always be “runts”!) **So it is important to regularly sift bowls to remove brood tubes and prevent the next generation from hatching and sharply increasing the density.** However, do not sift more often than 1x/week – it “stresses” the worms and they will not produce brood tubes.
    - Try to maintain about a 50:50 ratio of males to females. When females are absent or rare, males will become hermaphrodites i.e. develop ova. (We have a number of articles on this subject too).
    - For reference:

| <i>Capitella teleta</i>                                      | Males                                                             | Females                             |
|--------------------------------------------------------------|-------------------------------------------------------------------|-------------------------------------|
| Morphological sexual characteristics                         | 2 pairs of apposed dorsal chaetal spines in thoracic segments 8-9 | ova (visible through the body wall) |
| Average age until sexual maturity (at $15^{\circ}\text{C}$ ) | 8-10 weeks                                                        | 8-10 weeks                          |

- ❖ Label the bowl with: 1/ start date, 2/ most recent date sifted, 3/ number of sifts (e.g. 4<sup>th</sup> sift), and number of worms. Note: on first sift, carefully count number of males and females. OK to estimate after that.
- ❖ Since you have transferred the worms to a bowl with fresh mud, you can skip feeding that bowl for the week.

**V. Starting a new bowl/inducing metamorphosis (for sand bowls, refer to “Optional Culturing” under part III)**

- Fill a finger bowl halfway with SW. Gently place 1/2 teaspoon of mud on the bottom of the bowl with as little dispersal as possible (want a blob of mud with clear water).
- Add 40 larvae (do NOT exceed 40 animals):
  - ❖ Try to use larvae from a number of brood tubes (4-8 minimum) and from different bowls to ↑ genetic diversity.
  - ❖ Try also to use broods from large, healthy moms with large broods (“runts” tend to spawn “runts”).
  - ❖ Place 1-2 brood tubes from late larval stages (st. 7-9) in a 35mm uncoated dish with 0.2 μM SW and keep in the 19°C incubator until the larvae swim out from the brood tube (=hatch). CAUTION: Allow broods to naturally hatch, do not dissect larvae from the brood tube. (Mom can survive without food for a few days without mud, but try to minimize the number of days the mom is without food. It is best to use late stage broods (stage 6-9) that should hatch within a few days. If have early stage broods, place a little mud in the dish).
- Label the finger bowl with: 1/ “New Bowl”, 2/ number of larvae added (n), 3/ date, and 4/ your initials. Add notes regarding # of broods and from which bowl(s) broods were obtained in Animal Care notebook.
- Leave on counter at RT and do not move the bowl for at least 24 hours (allowing the larvae to settle and metamorphose).
- In 1-2 days, place in the 15°C incubator. Animals will develop more slowly, but will reach a larger size.
- For the 1<sup>st</sup> week, just change the SW, then after 2 weeks start feeding.
- After 4-5 weeks (or whenever the mud layer looks like it is getting too thick), do a ‘quick and dirty’ sift: follow the same steps as regular sifting, except instead of transferring worms from the sieve into a petri dish, squirt them right back into their finger bowl and make a small note of the date they were ‘sifted’ on the label. Before adding fresh mud and SW, do a quick check for brood tubes under the dissecting scope.
- When the new bowls are 8-10 weeks old (sexually mature), they should be moved to the 19°C incubator and sifted every 2 weeks along with the rest of the colony.
- Try to start 2-4 new bowls every week.

**VI. Brood Tube Dissection**

- Use fine forceps (Dumont #5) and dissecting microscope.
- Group the brood tubes by stages into separate 35mm dishes. (We have a reference of the stages in the lab.)
- Methods to determine the stage of the larvae:
  - ❖ Occasionally, you can see the larvae through clear sections (no sand/mud) of the brood tube.
  - ❖ Make a “window” by gently scraping away a small section of sand/mud.
  - ❖ Make a small tear in the brood tube with sharp forceps and dissect a couple of larvae out.
- For early stages (pre-swimming), it may be easier to dissect in a coated Petri dish (see “Coated Dishes” protocol) as these stages tend to get stuck to the bottom. Remember to rinse the coated dishes a few times with SW before using.
- Carefully dissect away the brood tube without harming the mom or larvae.
  - ❖ The most popular technique is to use blunt forceps to hold/steady the tube, then use a sharp one to pull away pieces of the brood tube, releasing the larvae.
- Return moms to their bowl(s).
- Once finish dissecting all embryos/larvae from the brood tube, “Clean” the larvae by transferring the larvae to a clean dish
- The larvae are now ready to be used for another application (e.g. fixation, antibody staining, etc.).

**VII. Colony Maintenance**

-Labeling on bowls. Each bowl should be labeled with: 1/ bowl start date, 2/ most recent sift date, 3/ which sift it is (e. g. 1<sup>st</sup> sift, 4<sup>th</sup> sift, etc.), 4/ number of worms, and 5/ your initials.

-What to do as adults start to age (around the 4<sup>th</sup> - 9<sup>th</sup> sift). Keep only the healthy worms, can combine bowls, preferably from the same start date. Get rid of bowls when most or all of the worms look old and/or unhealthy.

-It is important to keep records of the colony. There are two spread sheets: 1/ sift schedule for bowls being sifted every two weeks, and 2/ new bowls, to keep track of new bowls in 15°C incubator. Once move bowl to 19°C incubator, add to sift schedule sheet on date of first sift.

\*This protocol was developed from a compilation of information from 1) Sue Hill (see cappy care 10/01), 2) Jeanine M. Rosario (Rutgers University, see New Jersey culturing protocol), and 3) our own observations and experiments.