Timing the start of fertilization in *Littorina saxatilis*

Schedule and protocol

Day 1 (2nd September @10:30):

1. Collect ~150 snails from Saltö at the crab habitat (virgin females are Crab) but not in ANG. We expect to end up with 70-80 males as the sex ratio is 1:1.
2. Sex snails, measure shell size of males only and mark them with nail varnish.
3. Place males in damped Eppendorf tubes, individually.
4. Label tubes using FLs\_01, FLs\_02, …
5. Place tubes in 4°C temperature.

If there is enough time:

1. Take virgin females (~40 but depends on how many are available) out from the tanks.
2. Repeat steps 3, 4 and 5 for females.
3. Create pairs by matching labels of females and males based on the estimated optimal size ratio (OR = 0.3 on the natural logarithmic scale).

Otherwise next day.

Day 2:

1. Prepare n number of plastic spheres by filling up one third with sea water (record temperature). Same n for each group (one minute, five minute and 30 minute group).
2. Place one pair of snails per plastic sphere with the foot touching the bottom of the sphere.
3. Wait until the male (the marked snail) assumes the typical mating position and start timing. Total time of each experiment set is 2 hours.
4. Keep looking for any movements of the male and record time of dismount if this occurred before the mating pair was supposed to be manually interrupted.
5. Abort copulation.
6. Put the snails back in the tubes and store the females …
7. Repeat previous steps until n - 10 females have been used (10 females as a control that they were virgin).

Dissect males to confirm sex?

Then dissect females after 2-3 weeks and photograph brood pouch.

What about storing male/female/brood tissue, in case we want to check paternity or maybe just check whether miss-developing embryos are unfertilized?