**Protocol for fixation of Daphnia/parasite samples in formaldehyde:**

1. When you check experimental day on a daily basis, always keep one/two extra empty rack(s) easily accessible in the Daphnia room. These will be your “graveyard-racks”. After you have removed and counted all juveniles from the jars (if any), place the dead jars on the side, in one of those racks.

2. Please keep in mind: when deciding if a Daphnia can already be considered “dead” at the time of daily inspection, please double-check after placing it on the “graveyard-rack”, that the individual is stuck at the bottom, not moving its antennae, and also not moving its feeding apparatus (which can be harder to see, especially for small or young Daphnia). If the Daphnia is in a state of near-death, but clearly still able to move its limbs, I’d suggest putting it back in the experiment for the remaining day and wait until the next day to consider it dead (PS: sometimes the Daphnia can even survive 1-2 days in this state, so we shouldn’t argue that “it would have died today anyway”, we can’t say for sure.

3. Once you have finished your daily check, go back to the “graveyard-racks” to perform double-check, then look up on the list the matching number of the Eppendorf that should host each Daphnia. Order your Daphnia on the rack in the right order – to avoid making mistakes – then fetch in your Eppendorf boxes, the tubes that you need to fix these Daphnia (i.e. with the same number). I’d suggest putting these eppis on a rack for eppis, that you would also keep accessible in the lab. Order your eppis in the same order as your graveyard jars.  
  
4. Using a different glass pipette for each individual, retrieve each dead body and put it in the matching eppendorf: then, fill up the level of the medium in the eppi up to (or down to) **0.5mL** (PS: this will be important for later). Normally you can always adjust in the eppi using the glass pipette, this you should do with medium from the current jar.  
  
5. Now that all dead Daphnia have been transferred to eppis, take off the labels from your “graveyard” jars, and stick them in the current day’s page of your lab-notebook.  
  
6. Put on some gloves before handling the formaldehyde stock (!!). Take one of the formaldehyde stock jars (labelled 37%) from Uschi’s room, as well as a yellow pipette (100uL or 200uL), that can handle 50uL.  
  
Bring your rack of dead eppis to the clean-bench, and if possible only add the formaldehyde under the bench (& with gloves). Open all your eppis, then take **50uL** from the formaldehyde for each tube and drop it slightly above the eppi (this way, you can use only one pipette tip to fill up all eppis with formaldehyde), then close the lid. Repeat until all your eppis have received formaldehyde.  
  
NB: by adding 50uL of formaldehyde (already diluted at 37%), in eppis that contain 500uL of medium, you end up with a final concentration of 3.7% (~4%) formaldehyde, which is sufficient to preserve the Daphnia’s body intact, along with spores of Metschnikowia, allowing counting later on at the microscope.  
  
7. Bring your eppis back to a fridge at 4°C (preferably the one in the clean-bench room). I’d suggest to store your collected samples in a different box (or racks) than your empty eppendorfs, so that these ones can stay in the fridge at all times (for instance you can start with a rack, properly labelled with your name in the clean-bench room fridge - then later transfer them back to boxes, once you have a larger number of collected samples).  
  
  
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NB: we should start this whole process at the minimum, on the 9th day after the inoculation. Based on previous experiments with these strains of Daphnia and parasite, symptoms of infection generally appear around day 10-11, with earliest observation on day +9.  
Though I have never observed the presence of mature spores on day +8, we might choose to start this process already one day ahead (so that we’re sure that we won’t miss any potential infection).  
  
Any Daphnia that dies before day 8-after-inoculation, we will not be able to identify if it would have been successfully infected or not (Daphnia can recover up until the stage of ascus production), so you can simply discard the dead ones at this point of the experiment. Please already keep the labels in your notebook, though.