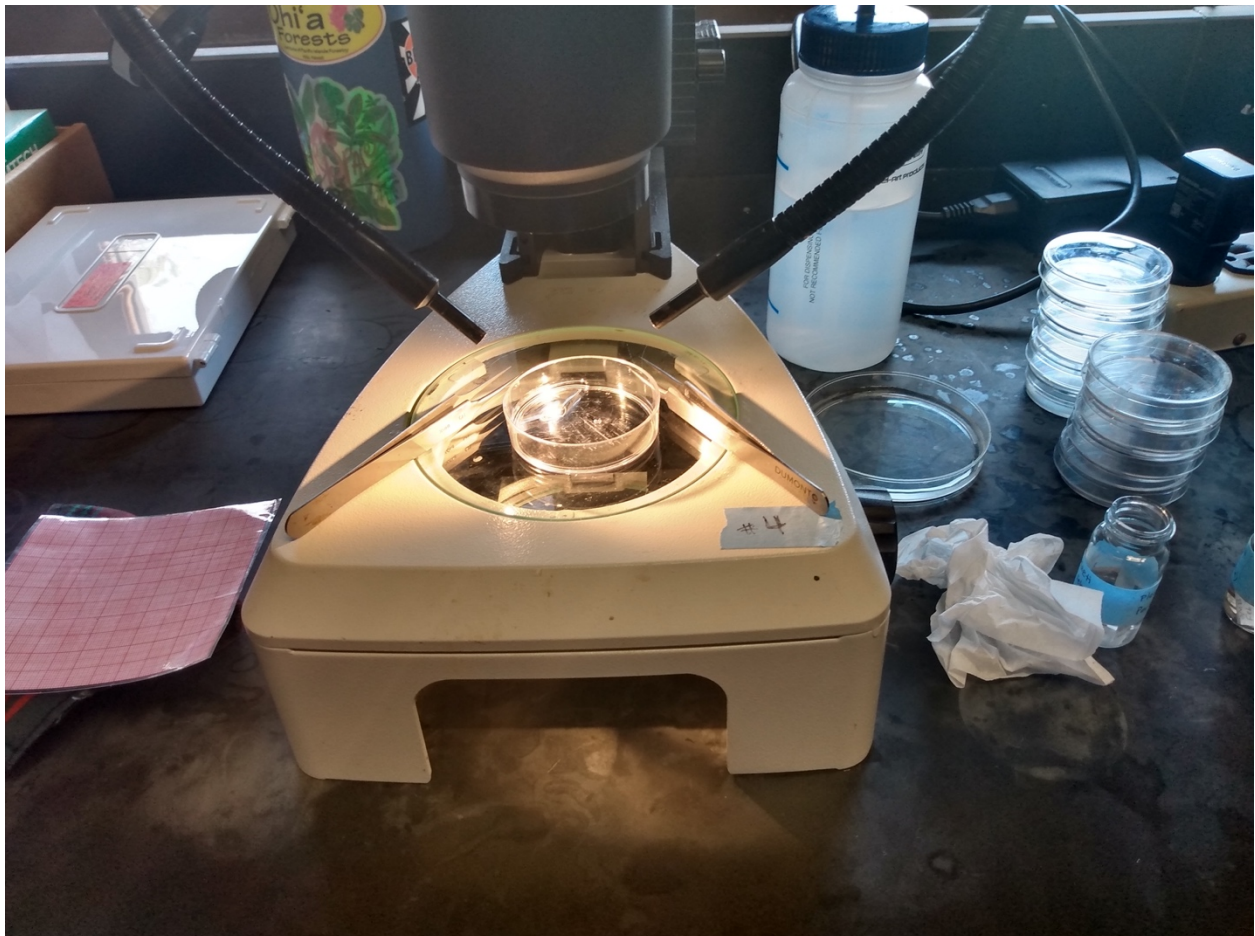


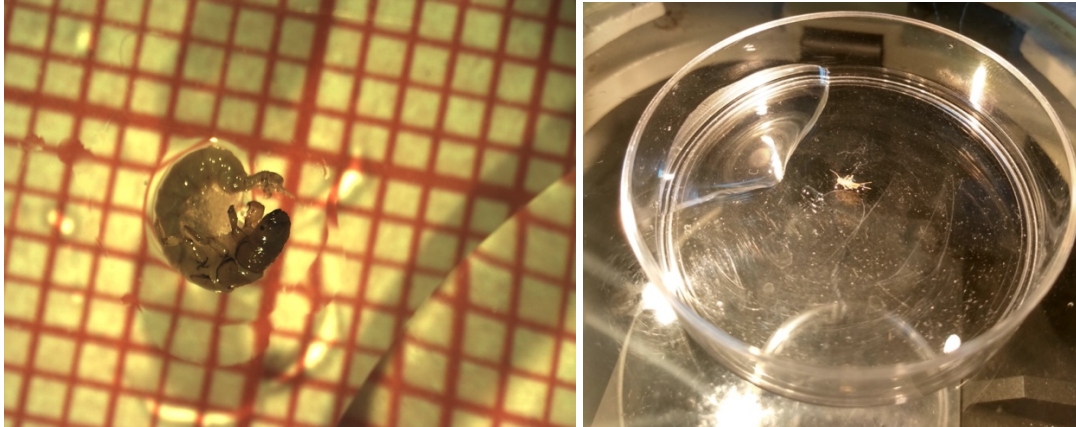
### Insect Gut Dissection Methods

**1. Set up:** Before you start, make sure that you have the following materials ready:

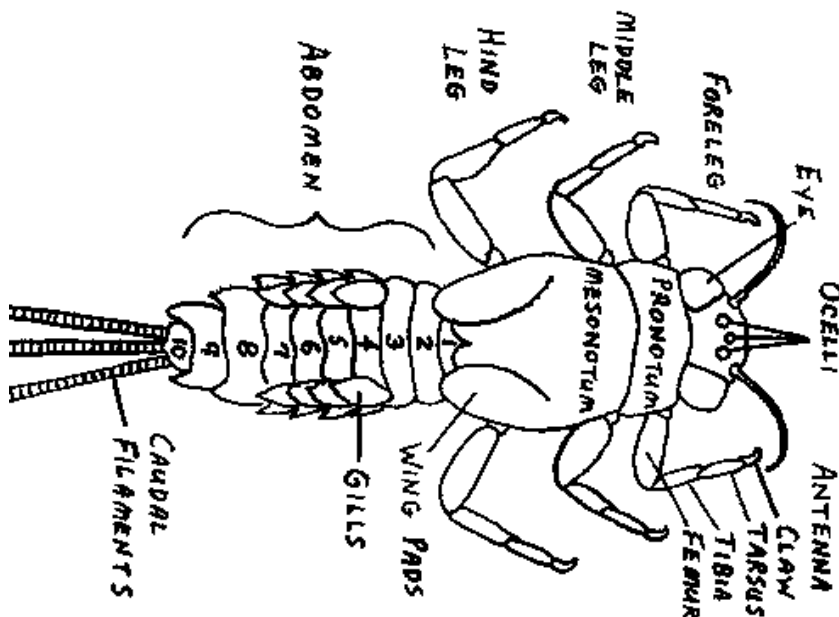
- squirt bottle with water
- 10 numbered small petri dishes
- 2 very fine tipped forceps
- millimeter paper or ocular micrometer to measure length of invert
- tissue for cleaning
- waste container (usually bigger petri dish) filled with water
- a dissecting microscope with gooseneck lighting



**2. Prepare invertebrate:** Once set up, squirt a little bit of water on the edge of your first petri dish. Place your invertebrate just outside of the water. Only analyze insect larva, not adults. Note: always make sure your invertebrate and gut content is moist. The light source is very hot and evaporation can happen fast.

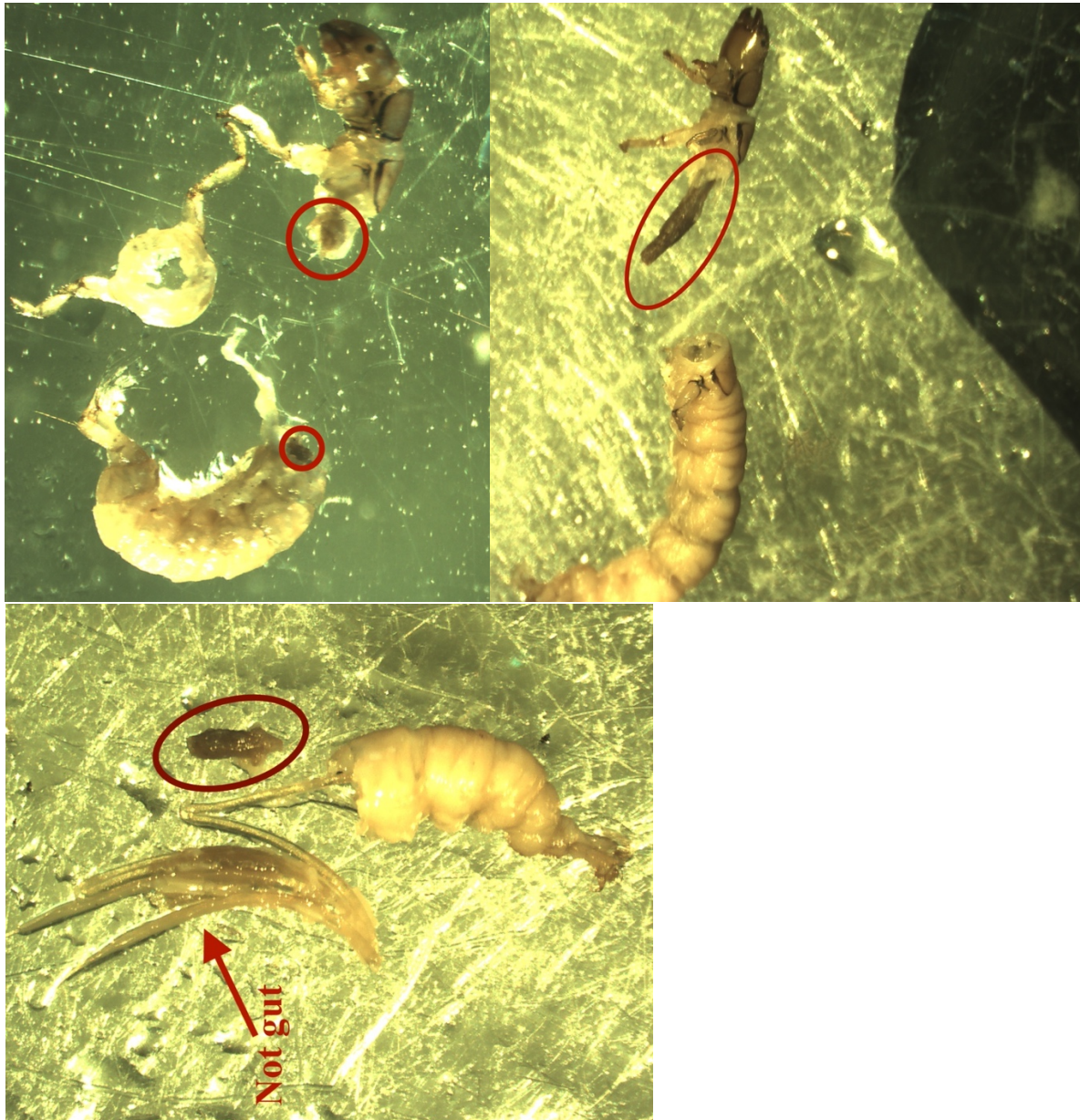


**3. Measure length:** Measure the length of the invertebrate from the top of the head (without antennas) until the end of the main body. Do not include cercis (caudal filaments) or caudal claws/gills. You can measure the length by using the millimeter paper or an ocular micrometer, if available. If you are using the ocular micrometer, make sure it is adjusted to the magnification that you are viewing the invertebrate at. Make sure you are familiar with the basic anatomy of your invertebrate before you are dissecting it so you know you are picking out all the pieces that do not belong to the gut.



**4. Remove gut:** Time to dissect the entire gut out from the body. First, remove the head by grabbing the head with one forceps and the end of the abdomen with the other forceps. Many things can happen here. If the gut comes out with the head (commonly for caddisflies and beetle larva), great! More likely only a piece of the gut will come with it. The most important part of the gut is the closest piece to the head (least digested material), so please take extra care of removing it. As soon as you separated the gut, place the gut into the water. Remove any material free of the gut tract immediately from the small petri dish into the larger waste container with water. To uncover the rest of the gut keep pulling on the outside body wall to remove more body segments, and push out the gut tract until the entire tract is exposed.

Below caddisfly example: Gut circled in red



Below beetle larva example: Gut circled in red



For mayflies and stoneflies the dissection is often a little different. The gut rarely comes off with the head. Nonetheless, first remove the head and the last abdomen segment with the tails. If any gut is removed, separate it. For the mid-section, I find “cutting” the insect open with your forceps (like a surgent) is the easiest. Approach it from the ventral side of the body and pull the sides of the body apart until it ruptures. You should see the gut displayed.

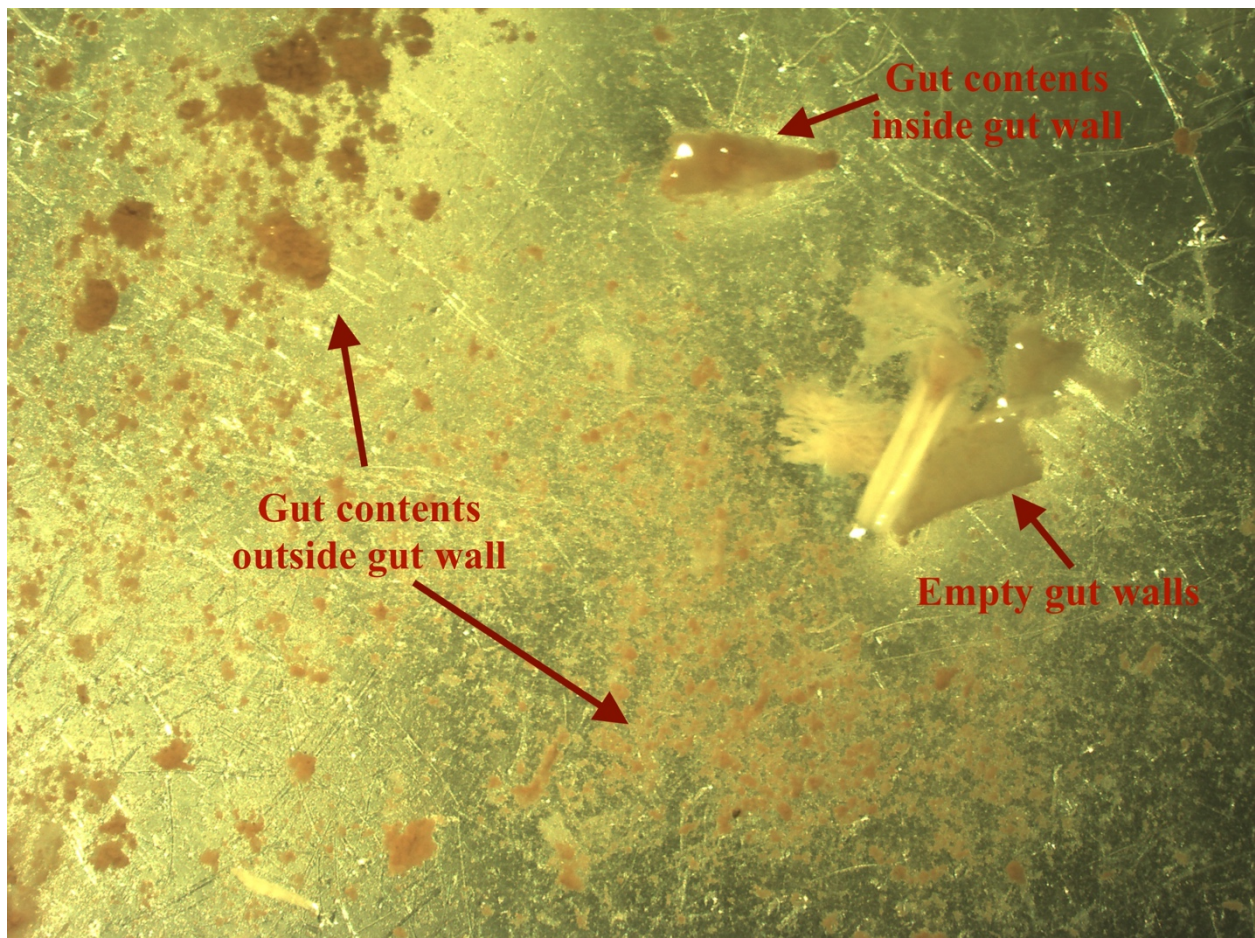
Below mayfly larva example: Gut where arrows point



Again, as soon as any gut is freed from the insect, place it in the water on the edge of the petri dish. As soon as you have a piece that does not have the guts, remove it from the petri dish and put it in your waste container filled with water. The water will help remove the trash. Wipe any excess material off with your tissue. Watch out for any loose gills, legs, claws, antenna.

Please note, if you are not finding a gut, that just means the gut is empty. Filled guts will always be a brownish tube. If you are not finding a brown tube, discard the whole insect, wipe your petri dish, and find another invertebrate.

**5. Empty gut contents:** Once only the gut is in your petri dish, remove all the contents. Depending on how tough the gut wall is, you can just squeeze out the contents out or tear open the wall and shake the contents out. Remove all the gut wall from the water and place it into your waste. At the end you should have only gut contents in your petri dish water. Scan again through the water and the rest of the petri dish ensure you do not have a piece of the dissected gut wall or insect in your water. Pay special attention if your insect is a predator and avoid mixing any predator material with its cut contents. You may see whole insects (e.g. midges) in predators.



**6. Final preparation before sonication:** Add more water from the squirt bottle into your petri dish. You do not need to fill it, just add enough to make sure that it will not dry out while you are dissecting other individuals. Place the petri dish cover on top to avoid evaporation.



I usually prepare ten petri dishes (i.e. ten insect guts) at one time, before I move on to the sonication process. Before sonication, make sure you have the appropriate filter sizes available. If you are dissecting an insect < 8mm the 13 mm diameter filters are great. Insects > 8 mm, use the 25 mm diameter filter. Also label your slides with:

Slide #  
Site  
Sampling Date  
3 letter taxa name  
Replicate number